DISSERTATION ON

"EXPERIMENTAL EVALUATION OF ORAL ANTI DIABETIC ACTIVITY OF WHOLE PLANT EXTRACT OF *Tephrosia purpurea* IN STZ-NICOTINAMIDE INDUCED DIABETIC RATS"

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CERTIFICATE

This is to certify that Dr Deepti.V.D, a Post Graduate student in the Department of Pharmacology has carried out the work titled **"EXPERIMENTAL EVALUATION OF ORAL ANTI DIABETIC ACTIVITY OF WHOLE PLANT EXTRACT OF *Tephrosia purpurea* IN STZ-NICOTINAMIDE INDUCED DIABETIC RATS"** under the guidance of DR. R. KAVITHA, M.D., PROFESSOR & HOD, Department of Pharmacology, towards the partial fulfilment of regulations laid down by The Tamilnadu Dr. M.G.R Medical University, Guindy, Chennai, Tamilnadu, India for the award of Doctor of Medicine (M.D.) in Pharmacology.

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DECLARATION

This is to declare that **"EXPERIMENTAL EVALUATION OF ORAL ANTI DIABETIC ACTIVITY OF WHOLE PLANT EXTRACT OF *Tephrosia purpurea* IN STZ-NICOTINAMIDE INDUCED DIABETIC RATS"** submitted by me for the Degree of M.D. is the record work carried out by me during the period of March 2017 to April 2017 under the guidance of Dr. R. Kavitha M.D., Professor and HOD of Pharmacology, Karpaga Vinayaga Institute of Medical Sciences and Research Centre and has not formed the basis of any Degree, Diploma, Fellowship, titles in this or any other University or other similar Institution of Higher learning.

Place: Chinnakolambakkam

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LIST OF ABBREVIATIONS

1.	STZ	–	Streptozotocin
2.	WHO	–	World Health Organization
3.	ADA	–	American Diabetes Association
4.	IDDM	–	Insulin dependent diabetes mellitus
5.	NIDDM	-	Non- Insulin dependent Diabetes Mellitus
6.	GDM	–	Gestational Diabetes Mellitus
7.	HbA1C	-	Glycosylated Haemoglobin
8.	IAPP	–	Islet Amyloid Polypeptide
9.	DKA	–	Diabetic ketoacidosis
10.	NKHS	-	Non – Ketotic Hyperosmolar state
11.	PARP	–	Poly ADP- Ribose polymerase
12.	ATP	–	Adenosine Triphosphate
13.	NAD	-	Nicotinamide diphosphate
14.	AST	–	Aspartate transaminase
15.	ALT	–	Alanine transaminase
16.	ALP	–	Alkaline Phosphatase
17.	HDL	-	High density lipoproteins
18.	LDL	–	Low density lipoproteins
19.	TP	–	*Tephrosia purpurea*
20.	BUN	–	Blood Urea Nitrogen
21.	CMC	–	Carboxy methyl cellulose
22.	ICR	-	Institute of cancer research

1. TITLE

EXPERIMENTAL EVALUATION OF ORAL ANTIDIABETIC ACTIVITY OF WHOLE PLANT EXTRACT OF *Tephrosia purpurea* IN STZ-NICOTINAMIDE INDUCED DIABETIC RATS

2. INTRODUCTION

INTRODUCTION

Diabetes mellitus is one of the major public health burden leading to increased mortality. It is a metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism due to abnormalities in insulin production[1]. DM is also characterised by insulin resistance and pancreatic β cell dysfunction. Other factors predispose to type 2 Diabetes Mellitus are genetic variation, ageing, sedentary life style and obesity. In 2000, there were around 171 million diabetes cases and it is estimated that the number will double by 2030. A leading non communicable disease with multiple aetiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world. It is estimated that more than 62 million people are currently diagnosed with diabetes mellitus and is rapidly attaining the potential epidemic in India[2]. It has been reported that India (31.7 million) topped the world with the highest diabetic patients followed by China (20.8 million) and US (7.7 million) [3].

Ancient literature has explained the use of various herbs in the treatment of diabetes mellitus. Many investigations of oral anti-hyperglycaemic agents of plant origin used in traditional medicine have been conducted and many plants have been found to show positive activity. Various animal models like genetic models, fat-fed diet model, and chemical induced diabetes model are available with their own advantages. STZ/Alloxan induced diabetic model is the one which is

commonly used in experiments. The emerging ideal model for anti-diabetic activity is STZ-Nicotinamide induced model since has the advantage of reduction in mortality[4]. Diabetes has become the trending disease; various folk medicines with several kinds of herbs are consumed and is also recommended for the management of Diabetes mellitus.

Tephrosia purpurea Linn is a pan tropical, polymorphic, perennial herb belongs to the family fabacae popularly known as Saraphunkha in Sanskrit, Purple tephrosia in English with medicinal properties. Aqueous and ethanolic extract of seeds has anti-hyperglycaemic activity. Though there are many studies available, lack of information on utility of whole plant extract of *Tephrosia purpurea* in treatment of diabetes and their safety in renal and hepatic profile. Hence the present study is designed to explore the anti-diabetic activity and its safety in STZ-Nicotinamide induced diabetic rats.

3. AIM AND OBJECTIVES

AIM AND OBJECTIVES

AIM:

The aim of the study was to evaluate the anti-diabetic activity of whole plant extract of *Tephrosia purpurea* on STZ – Nicotinamide induced diabetes in Albino Wistar Rats

OBJECTIVES:

Primary Objective:

- To study the antidiabetic activity of ethanolic extract of *Tephrosia purpurea* whole plant against STZ – Nicotinamide induced diabetes in Wistar rats

Secondary Objective:

- To assess the safety of ethanolic extract of *Tephrosia purpurea* whole plant on lipid profile, liver function and renal function test.

4. REVIEW OF LITERATURE

REVIEW OF LITERATURE

Definition:

As per WHO " The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances in carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both"[5]

ADA defined as Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, Insulin action or both.[6]

History:

Diabetes mellitus has been known since antiquity. During the ancient period, poor knowledge of anatomy, Pathophysiology and lack of diagnostic tools the disease remained uncertain.

Ancient literatures Papyrus also mentioned about the distinctive features of patients suffering from diabetes and various treatment modalities. In 1500 BC, Ebers Papyrus described about the patient suffering from excessive thirst, increased urination and they were treated by extracts of herbal plant. In 2000 BC, Kahun Papyrus mentioned just the title of a recipe for the "treatment of thirsty woman" but there is no text[7]. In 1st century AD Rufus of Epherus and Galen mentioned about the symptoms of Diabetes. During the 2nd century AD, the term

"Diabetes" was introduced by Aretaneus in the medical nomenclature. Diabetes in Greek verb (diabaino) which means "I pass through" [8]

Thomas Willis coined the term "Mellitus" in 17th century which means sweetness in the urine[9]. The glycogenic action of the liver and role of pancreas in the pathophysiology was discovered by Claude Bernard in 19th century[10]. In the year 1889, Oskar Minkowski and Joseph Von Mering demonstrated that Pancreas is important for the maintenance of glucose homeostasis that was the turning point[11]. In the 20th century Banting and Best after performing several experiments in the dog, they discovered insulin[12]. In the year 1922, first insulin was administered to human. The discovery of insulin saved millions of lives and in 1923 they were awarded Nobel Prize in medicine[13]. The quest for oral anti-diabetic drugs began in the backdrop of the discovery of insulin[14]. In the year 1926, anti-hyperglycaemic moiety guanidine was isolated from the herbal plant *Galega officinalis* that marked the journey of oral anti diabetic agents. First synthesis of dimethyl biguanide (Metformin) is attributed to Werner and Bell in 1922 and it was Sterne's work that studied the mechanism of metformin in detail[14]. It was serendipity when Janbon, a French physician and his colleagues confirmed hypoglycaemia in patients treated with P-Amino sulphonamide isopropyl thiodiazole for typhoid in 1942. Further establishment of activity this group of drugs was in the year 1946, Lobaitres and his colleagues explained that these drugs stimulate β cell release of insulin[15]. Thiazolidinedione were discovered

in late 1970 when clofibrates like Ciglitazone given to patients with type 2 DM was found to have hypoglycaemic activity. Acarbose was isolated in 1975 from the strains of actinoplanes species and synthesized in laboratory and found to be the inhibitor of α glucosidase[16].

Epidemiology:

Diabetes is on the rise, it is no longer a disease of predominantly rich nations. The prevalence of diabetes is steadily increasing everywhere, most markedly in low and middle - income countries due to urbanization and rapid change of life style. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980[17]. The worldwide prevalence of DM has risen dramatically over the past two decades and has nearly doubled from 4.7% to 8.5% since 1980 in the adult population.

Over 90% of the diabetic population are suffering from type 2 diabetes mellitus and only about 5% have type 1 diabetes mellitus. Type 2 Diabetes already causes 5 million deaths per year, mostly from cardiovascular diseases and by 2030 it is expected to be 7th leading cause of death globally[18]. Although the prevalence of both type 1 and type 2 DM is rising much more rapidly presumably because of increasing obesity, reduced activity because of industrialization and the aging of the population. The prevalence is highest in Middle East and North Africa[17].

DM is fast gaining the status of potential epidemic in India with more than 62 million individuals currently diagnosed with the disease. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India[19]. It is estimated that the prevalence of DM in rural population is one quarter that of urban population for India.

Classification of Diabetes Mellitus[20]

- Type 1 Diabetes mellitus (Juvenile onset diabetes)
  - Immune mediated
  - Idiopathic
- Type 2 Diabetes Mellitus (Adult onset diabetes)
- Type 3 Other types of Diabetes Mellitus
  - Genetic defects in the beta cell development or function: Mutation in MODY (1-6), mitochondrial DNA, Pro insulin/ Insulin Mutation, Mutation in other pancreatic islet regulator/Protein.
  - Genetic defects in insulin action: Type A insulin resistance, Leprachaunism, Rabson –Mendhall Syndrome, Lipodystrophy Syndrome.
  - Diseases of the pancreas: Pancreatitis, Cystic fibrosis, Hemochromatosis.

- Excess amount of counter regulatory hormones: Glucaganoma, Somatostatinoma, Cushing's syndrome, Aldosteronoma, Phaeochromocytoma.
- Infections: Congenital Rubella, CMV, Cox Sackie Virus.
- Rare autoimmune disorders: Anti-Insulin receptor antibodies, Stiff-person syndrome.
- Genetic syndromes: Down's syndrome, Laurence Moon Biedl syndrome, Huntington's chorea.

➢ Type 4 Gestational Diabetes Mellitus

Aetiology and Risk factors:

Diabetes either Type 1 or Type 2, has equally strong genetic and environmental risk factors, an interaction of which leads to the clinical expression of the disease. Type 1 or Insulin dependent diabetes mellitus (IDDM) is often genetically-associated and immune-mediated. Individuals with IDDM have an absolute deficiency in insulin secretion and can be identified by serological evidence of autoimmune-mediated destruction of pancreatic islets or by genetic markers. However, this form of diabetes only accounts for 5-10% of those with diabetes. Also known as juvenile-onset diabetes, the rate of β-cells destruction in this form of diabetes is usually rapid in infants and children. However, it can occur at any age, even as late as eighties or nineties in life. Markers responsible for this destruction include islet cell autoantibodies (ICAs), insulin autoantibodies (IAAs),

glutamic acid decarboxylase autoantibodies (GAD65), and autoantibodies to tyrosine phosphatase IA-2 and IA-2α[21]. One and more of these autoantibodies are present in 85-90% of individuals when fasting hyperglycaemia is initially detected. Possibility of some aspect of diet and viral infection triggering an autoimmune exposure causing specific destruction of β-cells of the pancreas has been proposed. There is another form of IDDM where the pathogenicity is less well understood and hence known as idiopathic diabetes. Individuals in this category usually have permanent insulinopenia but lack signs of autoimmunity. This form of diabetes is strongly inherited. Hormone replacement therapy is not absolutely necessary for survival in this case as the degree of β-cell dysfunction varies among individuals[22].

The most common type of diabetes, Type 2 Diabetes mellitus or Non-Insulin dependent Diabetes mellitus (NIDDM) accounts for 90-95% of those with diabetes and has more complex aetiopathology. Individuals in this category can either have predominant insulin resistance with relative insulin deficiency or predominant insulin secretory defect with insulin resistance. The aetiology of this form of diabetes is wide and complicated, ranging from abnormalities in lipoprotein metabolism, central or visceral obesity, to cardiovascular risk factors such as hypertension. However, pancreatic islets destruction does not occur in NIDDM. On the contrary, insulin resistance may cause patient to have normal or even higher level of insulin. This form of diabetes is always associated with

obesity. It's becoming more common in developed and developing countries, afflicting younger generations victimized by a global epidemic of overweight and obesity[23]. There is another type of diabetes diagnosed during pregnancy named gestational diabetes. Most of the cases resolve with delivery, but the condition may persist in some cases as unrecognized glucose intolerance may have begun before the pregnancy.

Risk factors[20]:

- Family history of diabetes
- Obesity (BMI > 25kg/m^2)
- Physical inactivity
- Race/ Ethnicity (Africo American , Latino, Native Americans, Asian American, Pacific Islander)
- Previously identified with Impaired fasting glucose, Impaired glucose tolerance or on HbA_1C 5.7-6.4%
- History of GDM or delivery of Big baby >4kg
- Hypertension (BP >140/90mm Hg)
- HDL Cholesterol level <35mg/dl ; Triglycerides level >250mg/dl
- History of polycystic ovarian syndrome
- Previous history of cardiovascular disease

ADA Diagnosis Criteria[6]

The American Diabetes Association (ADA) criteria for the diagnosis of diabetes are any of the following:

- A HbA1c level of 6.5% or higher; the test should be performed in a laboratory using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized or traceable to the Diabetes Control and Complications Trial (DCCT) reference assay, **or**
- A fasting plasma glucose (FPG) level of 126 mg/dl (7 mmol/L) or higher, **or**
- A 2-hour plasma glucose level of 200 mg/dl (11.1 mmol/L) or higher during a 75-g oral glucose tolerance test (OGTT), **or**
- A random plasma glucose of 200 mg/dl (11.1 mmol/L) or higher in a patient with classic symptoms of hyperglycaemia (i.e., polyuria, polydipsia, polyphagia, weight loss) or hyperglycaemic crisis

Normal Glucose Homeostasis:

Plasma glucose is maintained at a rather consistent value of approximately 90 mg/dl (5 mmol/l), with a maximal increase of not exceeding 165 mg/dl (9.2

mmol/l) after a meal[24] or a decrease down to not lower than 55 mg/dl (3.1 mmol/l) after exercise or a moderate 60-hour fast. Glucose can be from dietary source or is either from the gluconeogenesis in liver and kidney or the breakdown of glycogen (glycogenolysis) in liver. This glucose may be stored directly as glycogen through the process of glycogenesis in liver or may undergo glycolysis, which can be non-oxidative, producing pyruvate or oxidative, through oxidization of acetyl CoA to carbon dioxide and water in the tricarboxylic acid cycle or commonly known as Krebs cycle (Figure 1)[25].

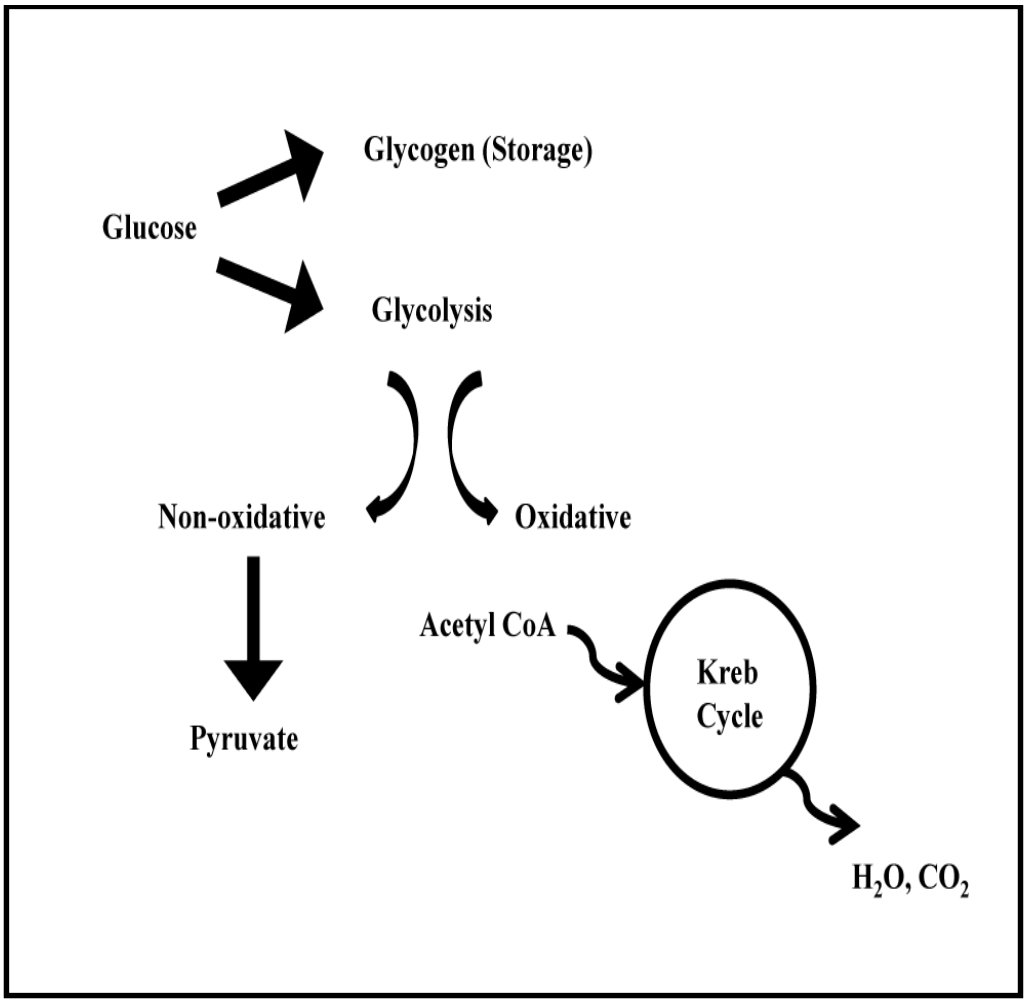


Figure 1. Fate of Glucose[25]

Glucose Homeostasis Regulators:

Insulin

This major regulator affects glucose metabolism both directly and indirectly. Its receptors are available in insulin-sensitive organs such as liver, kidney, muscle and adipose tissue. Activation of insulin signalling upon binding of insulin to insulin receptors causes suppression of gluconeogenesis in liver and

kidney[26], translocation of glucose transporter-4 (GLUT 4) from inner membranes to plasma membrane in liver, muscles and adipose tissue to increase glucose uptake[27], and inhibition of free fatty acid release into circulation[28]. As free fatty cid stimulates gluconeogenesis and reduce glucose transport into cells, release of insulin also indirectly regulates gluconeogenesis and glucose transport through free fatty acids. Besides, insulin promotes glycogen synthesis by inhibiting glucose-6-phosphatase (G6Pase) and glycogen phosphorylase while stimulating glycogen synthase[29]. Increased plasma glucose results in increase in plasma insulin while decrease in plasma glucose causes reduction in plasma insulin level as well.

Glucagon

Unlike insulin secreted from pancreatic β cells, glucagon is secreted from α-cells of the pancreas. Glucagon secretion is stimulated by hypoglycaemia whereas hyperglycaemia will inhibit its secretion. Glucagon acts exclusively on liver by activating glycogen phosphorylase and results in immediate glucose release. Further action of glucagon will be through stimulation of gluconeogenesis[30].

Catecholamines

Catecholamines are molecules that act as both hormone in blood circulation and neuromodulator in central nervous system. During stress and hypoglycaemia, catecholamine are released and they inhibit insulin secretion and action. In the liver, through β2-adrenergic receptors, they activate glycogen phosphorylase and

augment gluconegenesis[24]. In the kidney, they are potent stimulators of gluconeogenesis. In skeletal muscle, they reduce glucose uptake and stimulate glycogenolysis. They also activate lipase and result in lipolysis in adipose tissue to increase release of free fatty acid[31].

Growth Hormone and Cortisol

Both metabolic actions of growth hormone and cortisol are antagonistic to those of insulin. They increase secretion of gluconeogenic enzymes, reduce glucose transport and inhibit lipolysis. In addition, cortisol also impairs insulin secretion and therefore further debilitating insulin signalling.

Free Fatty Acids

As mentioned before, increased plasma free fatty acids will result in stimulation of renal and hepatic gluconeogenesis, inhibition of glucose transport in muscles and adipose tissue and competition with glucose as metabolic fuel[32].

Incretins

Incretins are hormones secreted by intestine in response to nutrients ingestion. Their main effect is to stimulate pancreas to release insulin after meals intake. Two incretin hormones were identified so far: gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP1). Both of them have short half-life due to rapid digestion by proteolytic enzyme known as dipeptidyl peptidase-4 (DPP-4).

Pathogenesis of IDDM

Type 1 diabetes mellitus is characterized by autoimmune destruction of insulin-producing β-cells in pancreas by alteration of CD4+ and CD8+ T cells and macrophages which causes infiltration in the islets of langerhans. [33]

Pathogenesis of NIDDM

Although NIDDM makes up most cases of diabetes mellitus, its pathogenesis remains unclear, most probably due to its heterogeneity. Two main factors account for the development of NIDDM, which are the genetic factors and the environmental influences. Studies have shown that most patients have a positive family history and the risk for developing NIDDM is increased up to 40% by having a first-degree relative with the disease[34]. Environmental factors such as physical inactivity, obesity and dietary habits may interact with genetic factors and increase the risk of developing diabetes.

β-cell dysfunction:

Compared to normoglycaemic subjects, impaired glucose tolerance (IGT) subjects secrete less insulin at any given glucose level[35]. Islet β-cells function declines progressively from IGT to complete glucose intolerance (CGI) which appears to be the reason why patients who are initially well controlled by a single oral hypoglycaemic agent require increasing dose or combined agents to maintain

glycaemic indices[36]. There are several possible causes that result in β-cells dysfunction. Glucotoxicity and lipotoxicity are conditions where islet β-cells are exposed to high glucose or free fatty acids levels chronically. Long-term exposure to high glucose and free fatty acid levels impair insulin secretion from β-cells[37]. Human islet amyloid polypeptide (IAPP) or amylin, is normally co-localized within the same secretory vesicles as insulin and co-released with insulin in response to glucose or non-glucose secretagogues[38]. Decreased IAPP release but increased islet amyloid deposit has always been found in NIDDM[39] and amyloid deposition has been proposed to decrease β-cell mass[40].

Insulin Resistance:

Instead of impaired insulin secretion, in some NIDDM patients, hyperinsulinemia coexists with hyperglycaemia, which is commonly associated with obesity and insulin resistance[41]. By using hyperinsulinemic-euglycemic clamp technique, obese and diabetic patients have been correlated to decreased responsiveness or diminished sensitivity in insulin-stimulated whole-body glucose disposal[42]. Reduced numbers of insulin receptors, impairments in insulin receptor substrate and PI3K are primarily related to insulin resistance. Besides genetic factor, acquired insulin resistance gains much attention for the prevention and progression of the disease. Owing to dyslipidaemia in obese patients, elevated free fatty acid levels are associated with non-alcoholic hepatic steatosis, insulin resistance, decrease in skeletal muscle glucose disposal and increased hepatic

glucose production[43]. Apart from decreasing β-cell function, chronic physiological increment in the plasma glucose concentration also leads to progressive insulin resistance in NIDDM[44]. However, to date, it is still unclear with regard to the relative contributions of pancreatic β-cell dysfunction and insulin resistance to the pathogenesis of NIDDM.

Complications of diabetes mellitus:

Diabetes predisposes patients to opportunistic infections, vascular and neurological complications. Based on its pathophysiology diabetes mellitus can be acute or chronic.

Acute complications

These include diabetic ketoacidosis (DKA) and non-ketotic hyper-osmolar state (NKHS). Diabetic ketoacidosis is seen primarily in individuals with type 1 diabetes mellitus while non-ketotic hyperosmolar state is prevalent in individuals with type 2 diabetes mellitus. The two disorders are associated with absolute or relative insulin deficiency, volume depletion and altered mental state[45]. In DKA, insulin deficiency is combined with counter-regulatory hormone excess with respect to glucagon, catecholamines, cortisol and growth hormone[46]. The decreased ratio of insulin to glucagon promotes gluconeogenesis, glycogenolysis and ketone body formation in the liver and also increases free fatty acid and

amino-acid delivery from fat and muscle to the liver[47]. Ketosis results from a marked increase in free fatty acid release from adipocytes due to increased lipolysis[48]. In DKA, nausea and vomiting are often present. Severe DKA may result in lethargy and central nervous system depression eventually leading into coma. Cerebral edema, an extremely serious complication, is seen most frequently in children[49].

Non-ketotic hyper-osmolar state is most commonly seen in elderly individuals with type 2 diabetes mellitus. Its most prominent features include polyuria, postural hypotension, and a variety of neurological symptoms including altered mental state, lethargy, seizure and possibly coma. Insulin deficiency and inadequate fluid intake are the underlying causes of NKHS. Hyperglycaemia consequent to insulin deficiency induces an osmotic diuresis leading to excessive intravascular volume depletion[50].

Chronic complications:

The chronic complications of diabetes mellitus affect multiple organs and are responsible for the morbidity and mortality associated with the disease. Chronic complications can be either vascular or nonvascular. Vascular complications are then subdivided into micro vascular (retinopathy, neuropathy and nephropathy) and macro vascular complications (coronary artery disease, peripheral vascular disease and cerebrovascular disease) [51]. Nonvascular

complications include gastroparesis, sexual dysfunction and skin changes. Diabetes mellitus is the most common cause of adult blindness, a variety of debilitating neuropathies, cardiac and cerebrovascular disorders[52].

Early in the course of diabetes, intracellular hyperglycaemia causes abnormalities in blood flow and increased vascular permeability. This reflects decreased activity of vasodilators such as nitric oxide, increased activity of vasoconstrictors such as angiotensin II and endothelin-1 and production of permeability factors such as vascular endothelial growth factor. In diabetes, arterial endothelial dysfunction seems to involve both insulin resistance specific to the phosphatidylinositol–3-OH kinase pathway and hyperglycaemia[53].

Diabetic retinopathy

Diabetic retinopathy occurs in 75% of all persons having diabetes for more than 15 years and is the most common cause of blindness. The risk of developing diabetic retinopathy or other micro vascular complications of diabetes depends on the duration and severity of hyperglycemia[54]. Diabetic retinopathy is classified into two stages, non-proliferative and proliferative. The non-proliferative stage appears late in the first decade or early in the second decade of disease and is marked by retinal vascular micro aneurysms, small haemorrhages in the middle layers of the retina, and cotton-wool spots and includes loss of retinal pericyte, retinal edema resulting from increased retinal vascular permeability, alterations in regional blood flow, and abnormal retinal microvasculature, all of which lead to

retinal ischemia[55]. Proliferative retinopathy is characterized by the formation of new blood vessels on the surface of the retina in response to retinal hypoxia. The newly formed vessels may appear at the optic nerve and/or macula and rupture easily, leading to vitreous haemorrhage, fibrosis and retinal detachment resulting in blindness[56].

Diabetic Neuropathy

The precise nature of injury to the peripheral nerves from hyperglycaemia is not known but likely related to mechanisms such as polyol accumulation, injury from advanced glycosylated end products and oxidative stress. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal and autonomic neuropathies. About half of all people with diabetes have some degree of neuropathy, which can be polyneuropathy, mono-neuropathy and/or autonomic neuropathy[57]. Polyneuropathy is the most common form of neuropathy in diabetes. There is loss of peripheral sensation which, when coupled with impaired micro vascular and macro vascular junction in the periphery, can contribute to non-healing ulcers, the leading cause of non-traumatic amputation[58]. There is thickening of axons, decrease in microfilaments, and capillary narrowing involving small myelinated or non-myelinated C-fibres. It can occur both from direct hyperglycaemia-induced damage to the nerve parenchyma and from neuronal ischemia leading to abnormalities of micro vessels, such as

endothelial cell activation, pericyte degeneration, basement membrane thickening and monocyte adhesion[59].

Pure sensory neuropathy is relatively rare and associated with periods of poor glycaemic control or considerable fluctuation in diabetes control. It is characterized by isolated sensory findings without signs of motor neuropathy. Symptoms are typically most prominent at night. Mono-neuropathy is less common than polyneuropathy and includes dysfunction of isolated cranial or peripheral nerves. Autonomic neuropathy can involve multiple systems, including cardiovascular, gastrointestinal, genitourinary, sudomotor and metabolic systems[60].

Diabetic Nephropathy

Diabetic nephropathy is defined by proteinuria greater than 500 mg in 24 hours, but this is preceded by lower degrees of proteinuria, or "micro albuminuria." Micro albuminuria is defined as albumin excretion of 30–299 mg per 24 hours[61]. Without intervention, diabetic patients with micro albuminuria typically progress to proteinuria with decreased glomerular filtration rate and end stage renal failure[62]. This progression occurs in both type 1 and type 2 diabetes. Dysfunction of the glomerular filtration is attributed to changes in synthesis and catabolism of various glomerular basement membrane macromolecules such as collagen and proteoglycans, leading to an increase in glomerular basement thickening[63]. Another possible mechanism to explain the increase in permeability

of the glomerulus is the increase in renal vascular endothelial growth factor (VEGF) levels observed in preclinical models of diabetes, since VEGF is both an angiogenic and a permeability factor[64].

Cardiovascular disorders

In diabetes mellitus there is marked increase in several cardiovascular diseases, including peripheral vascular disease, congestive heart failure, coronary artery disease, myocardial infarction. Cardiovascular disease is the primary cause of death in people with either type 1 or type 2 diabetes[65].

Macro vascular complications may be unaffected or even worsened by diabetic therapies. An improvement in the lipid profiles of individuals in the intensive group (lower total and low-density lipoprotein cholesterol, lower triglycerides) suggested that intensive therapy may reduce the risk of cardiovascular mortality. In addition to coronary artery disease, cerebrovascular disease is increased in individuals with diabetes mellitus[66]. Individuals with diabetes mellitus have increased incidence of congestive heart failure the aetiology of which may include factors such as myocardial ischemia from atherosclerosis, hypertension and myocardial cell dysfunction secondary to chronic hyperglycaemia. Low density lipoprotein particles found in type 2 diabetes are more atherogenic and are more easily glycated and susceptible to oxidation[67].

Hypertension

Hypertension is up to twice as common in patients with diabetes as in the general population. The central pathological mechanism in macro vascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system[68]. In response to endothelial injury and inflammation, oxidized lipids from low density lipoprotein particles accumulate in the endothelial wall of arteries. This results in the loss of elastic tissue from the walls of the medium and large arteries, which consequently become rigid. When elastic tissue is lost the arteries become increasingly less able to absorb the pressure wave, which is pumped into the circulation with every pulse, the pressure within the system therefore rises and the blood pressure goes up. High blood pressure in diabetes appears to hasten the slide to kidney failure; it accelerates the process of atherosclerosis, and is also associated with an increased mortality from stroke and Myocardial Infarction[69].

Infections

Individuals with diabetes mellitus exhibit a greater frequency and severity of infection[70]. The reasons for this include incompletely defined abnormalities in cell-mediated immunity and phagocyte function associated with hyperglycaemia

as well as diminished vascularization secondary to long-standing diabetes[71]. Many common infections are more frequent and severe in the diabetic population, whereas several rare infections are seen almost exclusively in the diabetic population. These include rhino-cerebral mucormycosis and malignant otitis externa, which is usually secondary to *Pseudomonas aeruginosa* infection in the soft tissue surrounding the external auditory canal[72]. Pneumonia, urinary tract infection, skin and soft tissue infections are all more common in the diabetic patients. The most common organisms causing infections in diabetic patients are *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*[73]. Diabetic patients have an increased rate of colonization of *S. aureus* in skin folds, nares and also have a greater risk of postoperative wound infections.

Management of NIDDM and IDDM

Management of diabetes and maintaining near-normal plasma glucose levels are of utmost importance in order to prevent the development of diabetic complications such as nephropathy, neuropathy, retinopathy, dyslipidaemia and cardiovascular diseases, which are comparatively more lethal. A number of therapeutic choices are available for the management of the disease; however, none are free of disadvantages. Pharmacological intervention remains the most effective way to control plasma glucose level but it is always associated with

unwanted side effects. Sulfonylureas initiate insulin release even when glucose level is low[74] and therefore are more likely to cause hypoglycemia[75]. Besides, as sulfonylureas stimulate insulin secretion, their effective use requires significant residual β-cell function[76]. Efficacy is better in patients shortly after diagnosis of NIDDM when most β-cell function is still preserved[77]. Thiazolidinedione's often cause weight gain which will further deteriorate insulin resistance and increase cardiovascular mortality risk e.g. pioglitazone[78]. The use of biguanides, such as metformin, is always associated with acidosis and severe gastrointestinal upset[79]. Over the past 30 to 40 years, studies using approaches ranging from epidemiological to interventional and molecular technologies have proven that regular exercise is effective in preventing and delaying metabolic diseases and its complications[80]. Unfortunately, sustained benefits are difficult to achieve due to incapability of human nature to adhere to exercise regimen. Also, evolutionarily humans have been driven to minimize energy expenditure and remain sedentary. As a result, dietary approach remains a crucial tool to achieve the goal of cost-effective management with minimal complications but maximal quality of life. Before the introduction of the therapeutic use of insulin, diet is the main form of treatment of the disease, and dietary measures included the use of traditional medicines which are mainly derived from plants[81]. Even now, approximately 80% of the third-world population is still dependent on traditional medicines. Metformin, the most prescribed and first-choice agent in NIDDM

pharmacotherapy, was derived from *Galega officinalis* (also known as Goat's rue or French lilac), a herb known for relieving symptoms of diabetes since the Middle Ages[82].

In addition, in recent years, a wealth of evidence has been obtained, correlating lower consumption of carbohydrate, saturated fat, processed food and higher consumption of fruits, vegetables, legumes, coffee, and tea with lower risk of diabetes and improved glucose and lipid metabolism. It is evident that plant-based foods are rich in phytochemicals known as polyphenols which include flavonoids, phenolic acids, lignans and stilbenes, which have been shown to improve glucose homeostasis at several organ sites, including the (1) gastrointestinal tract, which regulates carbohydrate digestion and glucose absorption, (2) endocrine pancreatic system, which secretes key regulatory hormones, insulin and glucagon, in response to abnormal glucose levels, (3) liver, where glucose synthesis, glycogen storage and breakdown are initiated, (4) insulin-sensitive peripheral tissues like skeletal muscle and adipose tissue, where glucose is metabolized for energy or stored for future use. Drugs used in the treatment of diabetes mellitus is given below (Figure 2)[20].

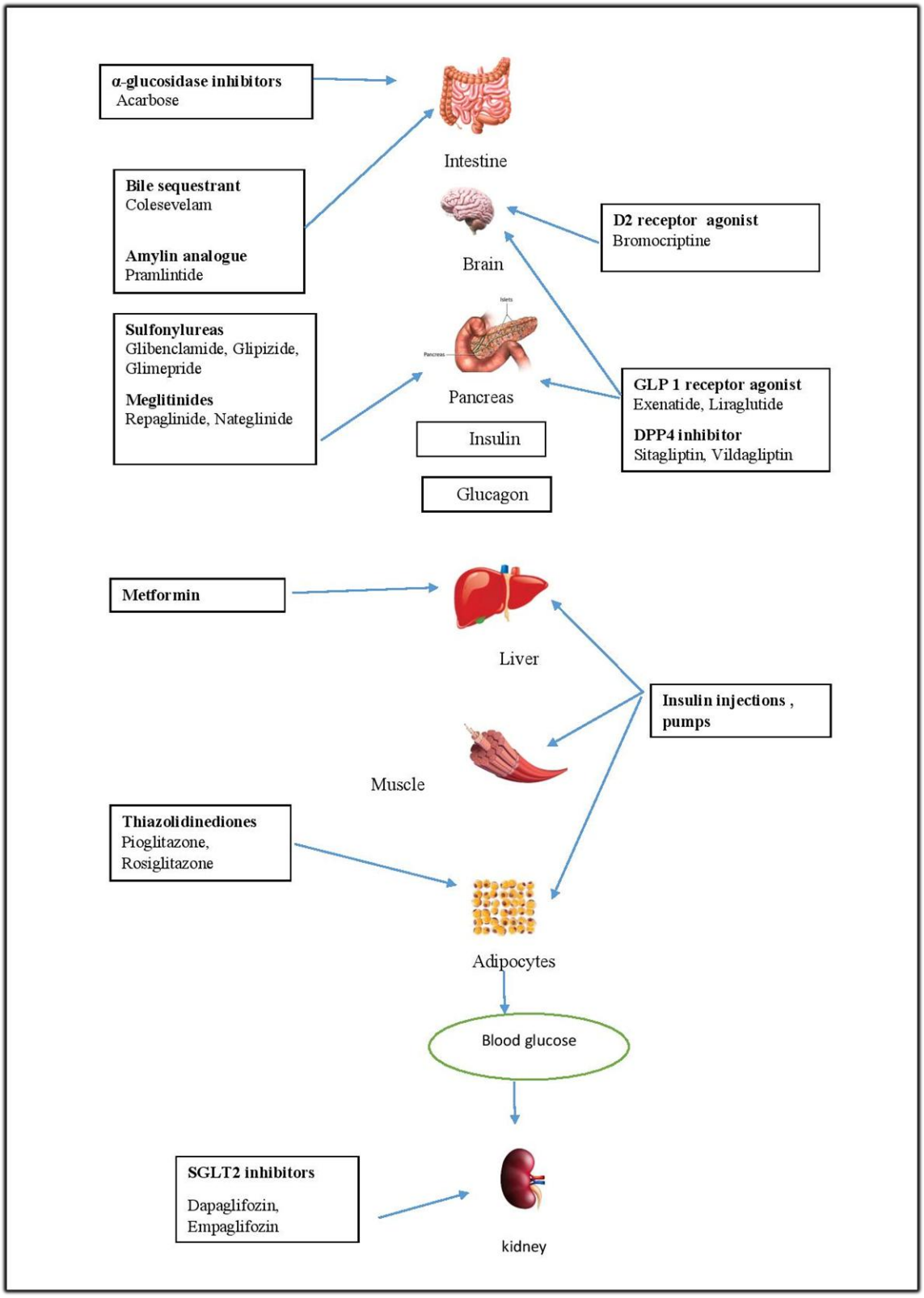


Figure 2. Drugs used in Diabetes Mellitus

Herbal medicines in the treatment of Diabetes Mellitus

Herbal medicine is the oldest form of healthcare known to mankind. It was an integral part of the development of modern civilization. Fossil records reveal the human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago which was evidenced by a burial site of a Neanderthal man uncovered in 1960[83]. Primitive man observed and appreciated the great diversity of plants available to him. Gradually, each tribe added the medicinal power of herbs in their area to its knowledge base. All cultures have long folk medicine histories that include the use of numerous plants.

The revival of interest in herbal medicines is firstly due to increased awareness of the limited horizon of synthetic pharmaceutical products to control major diseases and secondly due to the current widespread belief that 'green medicine' is safe and more accessible and affordable[84] than the costly synthetic drug many of which have adverse side effects. The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products especially from developed countries. According to a WHO estimate, about 80% of the world population relies on traditional systems of medicines for primary health care, where plants form the dominant component over other natural resources[85].

In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural

products with anti-diabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. Recent overwhelming attention to plant products and alternative medicine has encouraged plant chemists, pharmacologists, biochemists and molecular biologists to combine their efforts in a search for natural agents that can limit diabetes mellitus and its complications.

Animal models in diabetes:

1. Chemical induced Diabetes

1.1. Alloxan induced diabetes

Alloxan is most prominent chemical compound used in diabetogenic research. In research it is used for induction of Type 1 diabetes. Alloxan is a urea derivative which causes selective necrosis of the β- cells of pancreatic islets. It has been widely used to induce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used[86].

1.2. Streptozotocin (STZ) induced diabetes

Streptozotocin is a monofunctional nitrosourea derivative. First isolated from *Streptomyces achromogenes*[87]**.** It has been used alone or in combination with other chemotherapeutic drugs (vincristine, 5-fluorouracil, methyl-CCNU, procarbazine and 6-thioguanine) for the treatment of colorectal carcinomas and

other gastrointestinal cancers, but severe toxicity and myelosuppression were observed in most of the patients.

In the bacterial cells; it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The streptozotocin enters the pancreatic cell via a glucose transporter-GLUT2 causing DNA alkylation which further induces activation of poly ADP ribosylation and releases nitric oxide. This results in destruction of pancreatic β -cells leading to insulin dependent diabetes[88].

1.3. Dithizone induced diabetes:

Chemical name of dithizone is 8-(p- toluene- sulfonyl amino)–quinoline (8-TSQ). Dithizone is an organosulfur compound that acts as a chelating agent and forms complexes with lead, zinc and mercury. Zinc-chelating agent such as dithizone causes diabetes in laboratory animals. Dithizone has abilities to permeate membranes and to complex zinc inside liposomes with the release of protons, that can enhance diabetogenicity. When such complexing agents are added to lipid vesicles at pH 6 containing entrapped zinc ions, they acidify the contents of these vesicles. Such proton release occurs within the zinc-containing insulin storage granules of pancreatic beta-cells; solubilization of insulin would be induced which leads to osmotic stress and eventually the granule rupture and finally diabetes is induced[89].

1.4. Gold thioglucose induced diabetes:

Gold thioglucose is diabetogenic compound, which induces polyphagia and severe obesity induced Type -2 diabetes. It is derivative of sugar glucose. Gold thioglucose is precipitated with methanol and recrystallized with water and methanol. Gold thioglucose developed obesity induces diabetes in genetically normal mouse strains. Gold thioglucose treated DBA/2 (Dilute Brown Non-Agouti), C57BLKs, and BDF1 mice gained weight rapidly and significantly increase random plasma glucose level within 8-12 weeks. These mice showed impaired insulin secretion, mainly in early phase after glucose load and reduced insulin content in pancreatic islets[90].

1.5. Monosodium glutamate induced diabetes:

It is most abundant naturally occurring non- essential amino acid and freely soluble in water. Monosodium glutamate causes a very large insulin response after ingestion. It is developed glycosuria in both male and female mice but not induced polyphagia. Within 29 weeks level of glucose concentration in blood, total cholesterol and triglyceride were higher[91].

2. Virus Induced Diabetes

Juvenile- onset diabetes mellitus may be due to virus infections and beta-cell specific autoimmunity. In 1960s Gamble and co – workers reported newly diagnosed juvenile- onset diabetes (Type- 1) due to viral infections. At present

time two viruses are reported first is D- variant of encephalomyocarditis (EMC-D) and another is Coxsackie virus[92].

2.1. D- Variant Encephalomyocarditis

EMC- D virus can infect and destroy pancreatic beta cells in certain inbred strains of mice and produce insulin dependent hyperglycemia. Pre-treatment with a potent immunosuppressive drug, cyclosporine-A increases severity and incidence of diabetes in ICR Swiss mice. In 1992 Utsugi et al demonstrated the clone of EMC-D virus known as NDK25. Intraperitoneal injection of NDK25 develops non- insulin dependent diabetes mellitus[93].

2.2. Coxsackie Viruses

Coxsackie viruses are also a possible cause of diabetes in mice; it can infect and destroy pancreatic acinar cells while leaving the adjacent islets of langerhans intact. Coxsackie B4 virus is strongly associated with the development of insulin-dependent diabetes mellitus in humans. Diabetes induced by Coxsackie virus infection is a direct result of local infection leading to inflammation, tissue damage, and the release of sequestered islet antigen resulting in the restimulation of resting auto reactive T cells, further indicating that the islet antigen sensitization is an indirect consequence of the viral infection[94].

3. Hormone Induced Diabetes

3.1. Growth hormone induced diabetes

Growth hormone has long distinguished history in diabetes, with possible participation in the development of renal complications. Repeated administration of growth hormone in cats and adult dogs induces diabetes with all symptoms of diabetes including severe ketonuria and ketonemia. More prolonged administration of growth hormone produced permanent diabetes, there was loss of pancreatic islets tissues and of beta cells and only traces of insulin could be extracted from pancreas[95].

3.2. Corticosteroid induced diabetes

Corticosteroid used to reduce inflammation can lead to diabetes, which is called steroid diabetes. The most common glucocorticoids which causes steroid diabetes are prednisolone and dexamethasone. Glucocorticoids oppose insulin action and stimulate gluconeogenesis, especially in the liver, resulting in a net increase in hepatic glucose output and induce insulin resistance, hyperglycemia, and hyperlipidemia[96].

4. Genetically Induced Diabetes

The AKITA mouse was derived in Akita, Japan from a C57BL/6NSlc mouse with a spontaneous mutation in the *insulin 2* gene preventing correct processing of pro-insulin. This causes an overload of misfolded proteins and

subsequent ER stress. This results in a severe insulin-dependent diabetes starting from 3 to 4 weeks of age, which is characterized by hyperglycaemia, hypoinsulinaemia, polyuria and polydipsia. Untreated homozygotes rarely survive longer than 12 weeks. The lack of beta cell mass in this model makes it an alternative to streptozotocin-treated mice in transplantation studies. It has also been used as a model of type 1 diabetic macro vascular disease and neuropathy[88]. In addition, this model is commonly used to study potential alleviators of ER stress in the islets and in this respect models some of the pathology of type 2 diabetes.

STZ – Nicotinamide induced diabetes – Emerging model

Administration of both Streptozotocin (STZ) and Nicotinamide (NA) has been proposed to induce experimental diabetes in the rat. STZ is well known to cause pancreatic β-cell damage, whereas NA is administered to rats to partially protect insulin-secreting cells against STZ. STZ is transported into β-cells via the glucose transporter GLUT2 and causes DNA damage leading to increased activity of poly ADP-ribose polymerase (PARP-1). However, exaggerated activity of this enzyme results in depletion of intracellular NAD^+ and ATP, and the insulin-secreting cells undergo necrosis. The protective action of NA is due to the inhibition of PARP-1 activity. NA inhibits this enzyme, preventing depletion of NAD^+ and ATP in cells exposed to STZ. Moreover, NA serves as a precursor of NAD^+ and thereby additionally increases intracellular NAD^+ levels. The severity

of diabetes in experimental rats strongly depends on the doses of STZ and NA given to these animals. Therefore, in diabetic rats, blood glucose may be changed in a broad range from slight hyperglycaemia to substantial hyperglycaemia compared with control animals. Similarly, blood insulin may be slightly decreased or substantial hypoinsulinemia may be induced. In vitro studies demonstrated that the insulin-secretory response to glucose is attenuated in STZ-NA induced diabetic rats compared with control animals. This is due to reduced β -cell mass as well as metabolic defects in the insulin-secreting cells. Results of numerous experiments have demonstrated that this model of diabetes is useful in studies of different aspects of diabetes[97].

Plant Profile

Botanical name of the plant is ***Tephrosia purpurea*** which belongs to the family Leguminosae (Fabaceae). Common name of the plant is Wild Indigo, Saraphunkha. In Tamil it is called as **கொள்ளுக்காவேலை.** *Tephrosia purpurea* is a self-generating erect or spreading perennial herb found throughout India. It can be found as an ingredient in traditional herbal formulations.

Figure 3. *Tephrosia purpurea* (Linn) Pers.

Tephrosia purpurea is a small shrub that grows up to 1.5 meters tall. It has bi-pinnate leaves with 7 to 15 leaflets, the terminal leaflet being solitary.

Geographical Distribution

Tephrosia purpurea occurs naturally in grassy fields, waste places and thickets, on ridges and along roadsides, in Java. In Hawaii, it grows near the seashore. *Tephrosia purpurea* is native to tropical Asia, and is found from India and Sri Lanka to southern China and through South-East Asia to tropical Australia and the Polynesian Islands. It is now naturalized and cultivated pan tropically. *Tephrosia purpurea* grow at an altitude up to 400 m altitude, it generally grows at low altitudes but may be found to 1300 m altitude. It prefers dry, gravelly or rocky and sandy soils, but in India it grows well on loamy soils[98].

Ethno botanical uses of *Tephrosia purpurea*

- The plant has thermogenic, anti-helminthic, digestive, laxative, diuretic, uterine tonic, anti-inflammatory and antipyretic property
- The roots are useful in various infectious and inflammatory skin diseases. Some of its well - known use are in acne, boils, Asthma.
- The leaves are useful in gastrointestinal and genitourinary disorders.
- The seeds are also useful in skin diseases.[99]

Uses as folk medicine

Tephrosia purpurea was widely used as folklore medicine in most of the countries. Commonly it is used for cough, Chest tightness, bilious febrile attacks, and obstructions of spleen, liver and kidney. The leaves of *Tephrosia purpurea* was used to purify the blood, and used to cure for boils and pimples. Roots of *Tephrosia purpurea was* used for dyspepsia and chronic diarrhoea by the traditional healers. Infusion of seeds used as cooling medicine and decoction of pounded leaves used for snake bites.

In Ceylon, *Tephrosia purpurea* roots are used as anti-helminthic for children. Decoction of roots used as nematocide for treatment of *Toxocaracanis* larvae which causes lung disease. The plant is also used for colic, diarrhoea and dyspepsia. Fresh root-bark, ground and made into a pill, mixed with a little black pepper, used for obstinate colic.

In Indian medicine, *Tephrosia purpurea* is one of the common ingredients of various formulations which are used in the treatment of bilious febrile attacks, liver and splenic affections, cirrhosis and hepatitis. The oil obtained from the seeds of *Tephrosia purpurea* was applied topically to cure scabies, eczematous itching, and other skin eruptions. The plant was used to treat piles, syphilis and gonorrhoea by the local healers. In Punjab, infusion of seeds considered cooling agent. In some part of India, the plant seed was used as a substitute for coffee and as insect repellent[100].

Phytochemical review of Tephrosia purpurea:

- Chaudhari *et al.*, 2012, demonstrated regarding the presence of glycosides, various phytochemical constituents especially flavonoids in *Tephrosia purpurea*[101].
- Chang *et al.*, 2000, isolated three novel flavonoids, (+)-tephrorins A and B and (+)-tephrosone, from *Tephrosia purpurea* leaves extract[102].
- Venkata Rao and RangaRaju, 1984, isolated a flavanone, named as purpurin and identified as 2, 3 dihydrosemiglabrin from the seeds of *Tephrosia purpurea*[103].
- Gupta et al., 1980, isolated and characterized ten unusual and closely related flavonoids from the roots of *Tephrosia purpurea*[104].

Studies in accordance with our study:

- Pavana et al, 2007, demonstrated the anti-hyperglycemic activity of aqueous extract of *Tephrosia purpurea* seeds in streptozotocin induced diabetic rats. Profound alterations in the concentrations of blood glucose, lipids and lipoproteins were observed in diabetic rats[105].
- Hutchings and Van Staden, 2012, evaluated the ethanolic extract of plant Tephrosia purpurea shows anti-hyperglycemic activity against high fat diet

Wistar rat's model. A decrease in total cholesterol level of rats compared to hyperlipidemic control was observed[106].

- Pavana *et al.*, 2009, studied and stated that, aqueous seed extract of *Tephrosia purpurea* has potent anti-hyperglycemic and antioxidant effects in Streptozotocin-induced diabetic rats[107].
- Pavana *et al.*, 2007, demonstrated that *Tephrosia purpurea* leaf extract has prominent anti-hyperglycemic and anti-hyperlipidemic effects in Streptozotocin induced diabetic rats[108].
- Sayad Mustak, 2012, made a comparative study of *Tephrosia purpurea* (Linn) leaves and Lovastatin on cholesterol level of hyperlipidemic Wistar rats and it was found to be significant decrease in total cholesterol level of rats when compared to hyperlipidemic control which was equipotent as that of Lovastatin[109].
- Sangeetha and Krishnakumari, 2010, documented the hepatoprotective role of ethanolic extract of the root of *Tephrosia purpurea* against CCl_4 induced oxidative damage in the liver of rats[110].
- Khatria *et al.*, 2009, reported the hepatoprotective activity of aqueous–ethanolic extract of *Tephrosia purpurea* aerial parts (100, 300 and 500 mg/kg/day) against thioacetamide induced hepatotoxicity in Wistar rats[111].

There are several studies proved the various properties of different parts of *Tephrosia purpurea* such as anti-hyperglycemic, hepatoprotective

and lipid peroxidative effects. But none available on the whole plant extract of *Tephrosia purpurea* for anti-hyperglycemic activity and there are also no studies available on the Reno protective effect of *Tephrosia purpurea*.

Other studies related to Pharmacological activities of *Tephrosia Purpurea*:

- Rajaram *et al.,* 2015, report that the roots of *Tephrosia purpurea* showed neuroprotective activity against haloperidol induced Parkinson's disease in rats[78].
- Manjula *et al.,* 2013, documented the anthelmintic activity of aqueous and methanolic leaf extract of *Tephrosia purpurea* using *Pheretima posthuma* and the results showed that the anthelmintic activity was comparable to the standard Albendazole[112].
- Ashok Kumar *et al.,* 2012, explored the diuretic potential of *Tephrosia purpurea* plant extract on Wistar rats[113].
- Gopalakrishnan *et al.,* 2010, evaluated the anti-inflammatory and analgesic activity of ethanolic extract of *Tephrosia purpurea* aerial and root, which showed dose dependent activity against both acute and chronic models[114].
- Rumit Shah *et al.,* 2010, reported the hydro alcoholic extract of *Tephrosia purpurea* showed antioxidant activity by inhibiting DPPH and hydroxyl radical, nitric oxide and super oxide anion scavenging, hydrogen peroxide scavenging, and reducing power activities[115].

- Damre *et al.,* 2003, reported the cellular and humoral immunomodulatory activity of the flavonoid fraction of *Tephrosia purpurea in-vivo*[116].

Pharmacology of Glibenclamide – Standard drug

Glibenclamide is a potent second-generation sulfonylurea drug that improves glucose control by acting both on insulin secretion and on insulin action. The predominant effect of sulfonylureas was thought to be on insulin secretion, while the effect on insulin sensitivity may be mediated either through the improvement of metabolic control or via a direct peripheral effect[117]. The mechanism of action of glyburide seems to be initiated by the linkage of drug molecules with surface receptor in the β-cell surface and subsequent reduction of conductance of the ATP-sensitive K^+ channels[118]. The reduced K^+ efflux determines membrane depolarization and influx of Ca^{++} through Ca^{++} channels that eventually determine insulin secretion. In recent years increasing evidence has suggested a possible role of glibenclamide on insulin action at the level of different organ/tissues. Whether this drug effect is due to a direct, specific mechanism or is mediated by an improvement in glycemic control and consequently via a reduction of glucose-determined insulin resistance (glucose toxicity) is at present unknown. Finally, new interest in this class of drugs and in any other procedure that restores co-secretion of C-peptide and insulin (like whole

pancreas or islet of Langerhans transplantation) has been triggered by a number of recent reports showing a positive effect of C-peptide (at a chronic concentration as low as ~0.5 ng/ml) on the development of diabetic complications, this effect being mediated by activation of the Na^+/K^+ ATPase pump[118].

5. MATERIALS AND METHODS

MATERIALS AND METHODS

Plant Materials

Collection & Identification

The whole plant, *Tephrosia purpurea Linn.* was collected from the road side of Erode, Tamilnadu, during the month of October. It was authenticated by Prof.R.Duraisamy, Pharmacognosist and the voucher specimen (NCP/Phcog/2016/0202) has been retained, for future reference in the herbarium of Pharmacognosy department, Nandha College of Pharmacy, Erode, India.

Extraction of Plant Material

The collected *Tephrosia purpurea*, was washed in running tap water to remove the soil debris, shade dried and grounded using mechanical blender to get coarse powder. The 200gm of coarsely powdered *Tephrosia purpurea* whole plant was soaked in one litre of ethanol (90%) in a tightly sealed flat bottom flask at room temperature, protected from sun light for 72 hrs with occasional shaking. After 72 hrs the mixture was filtered through muslin cloth and the solvent was

evaporated by rotary evaporator at 40^{o}C to get dry mass. The dried ethanolic extract of *Tephrosia purpurea* was stored in desiccators and used for further pharmacological studies.

Animals

Wistar albino rats of either sex weighing between 180 – 200 gms were used for this study. The animals were obtained from King's Institute, Guindy and was housed in animal house, Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Kancheepuram. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (1818/GO/Ere/S/15/CPCSEA) and were in accordance with the Institutional ethical guidelines.

Experimental Induction of Diabetes in Rats

Diabetes was induced experimentally in 12 hour fasted rats by a single intra-peritoneal injection of Streptozotocin (50mg/kg) dissolved in 0.1M of citrate buffer (pH 4.5), followed by intra peritoneal administration of Nicotinamide (120

mg/kg) after 15 minutes. Since STZ is capable of inducing fatal hypoglycaemia due to sudden marked release of insulin from the pancreas, the rats that had been administered STZ were provided after 6 hr with a 10% glucose solution orally for 24 hr continuously so as to prevent hypoglycaemia. After 72 hr, rats with a blood glucose concentration above 200 mg/dl were considered to be diabetic and were used for further diabetic studies.

Experimental Design

After the successful induction of experimental diabetes, the rats were divided into five groups each comprising a minimum of six rats (Table 1).

Table 1 – Study animal groups

Group I	Normal rats received 0.1 % Carboxyl Methyl Cellulose Solution (1mg/kg) as vehicle through oral route.
Group II	Rats with STZ – Nicotinamide induced diabetic that were left untreated.
Group III	Rats with STZ – Nicotinamide induced diabetic that were treated for 30 days with orally administered Glibenclamide (5mg/kg)
Group IV	Rats with STZ – Nicotinamide induced diabetic that were treated for 30 days with orally administered ethanolic extract of *Tephrosia purpurea* (200mg/kg).
Group V	Rats with STZ – Nicotinamide induced diabetic that were treated for

	30 days with orally administered ethanolic extract of *Tephrosia purpurea* (400mg/kg).

Blood glucose levels were measured at different time intervals (0, 5th, 10th, 15th and 30th day) during the study. At the end of experimental period, the rats were fasted overnight, anaesthetized with Pentobarbitone sodium and the blood was collected by retro-orbital puncture in non heparinized tubes. To obtain serum, blood samples were placed at room temperature for 30 minutes and centrifuged at 3000 X *g* for 10 minutes and the supernatant were taken for the determination of Lipid profiles, Liver function (AST, ALT, and ALP) and Kidney function test.

Determination of Blood Glucose

1.) Glucometer was set out and a test strip, a sterile blade and an alcohol prep pad were arranged.

2.) The rat tail was clean with alcohol to prevent infection.

3.) Glucometer was turned and placed a test strip in the machine when the machine is ready. The indicator was observed to place the blood on strip.

4.) The tip of the tail was cut with sterile blade to obtain a drop of blood.

5.) The blood drop was placed on or at the side of the strip.

6.) With in few seconds after placing the blood on strip, the blood glucose levels was displayed.

Blood glucose levels were measured at different intervals using one touch glucometer.

Determination of Lipid Profile

Total cholesterol

Total cholesterol in serum was determined by a colorimetric method. The assay principle is based on enzymatic hydrolysis and oxidation of cholesterol and the indicator compound, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The reagents consisted of 4-aminoantipyrine (0.03 mmol/l), phenol (6 mmol/l), peroxidase (≥0.5 U/ml), cholesterol esterase (> 0.15 U/ml), cholesterol oxidase (> 0.1 U/ml) and pipes buffer (80 mmol/L pH 6.8). The serum sample (10 µl) was mixed with 1 ml of reagent, incubated at 37ºC for 5 min, and absorbance measured at 500 nm against the reagent blank[119]. The cholesterol standard was 5.17 mmol/l (200 mg/dl). The concentration of total cholesterol in the sample was calculated by

Total cholesterol = ΔAsample/ ΔAstandard x concentration of standard.

Triglycerides

Serum triglycerides (TG) were determined by a colorimetric method. The assay principle is based on the enzymatic hydrolysis of TG with lipases and the

indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic activity of peroxidase[120]. The enzyme reagent consisted of 4-aminophenazone (0.5 mmol/l), ATP (1.0 mmol/l), lipases (≥150 U/ml), glycerol-kinase (≥0.4 U/ml), glycerol-3-phosphate oxidase (≥1.5 U/ml), peroxidase (≥0.5 u/ml). The serum sample (10 µl) was mixed with 1000 µl of enzyme reagent, incubated at 37oC for 5 min and absorbance measured at 500 nm against the reagent blank. The TG standard was 200 mg/dl (2.29 mmol/l). The concentration of TG in the serum was calculated by

Total triacylglycerides =ΔAsample / ΔAstandard x concentration of standard.

HDL Cholesterol

Serum HDL cholesterol was determined by a colorimetric method. The assay principle is based on the following: the low density lipoproteins (LDL and VLDL) and chylomicron fraction is precipitated quantitatively by the addition of phophotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined. The precipitation reagents consisted of phosphotungstic acid (0.55 mmol/l) and magnesium chloride (25 mmol/l). The serum sample (200 µl) was mixed with 500 µl of precipitation reagent and centrifuged at 4000 rpm for 10 min. The supernatant (100 µl) was mixed with reagent (CH 200 1 ml), incubated at 37oC for 5 min and absorbance measured at 500 nm against the reagent blank[121].

The cholesterol standard was 200 mg/dl (5.17 mmol/l). The concentration of cholesterol in the supernatant was calculated by, HDL Cholesterol = ΔAsample / ΔAstandard x concentration of standard.

LDL & VLDL Cholesterol

Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were calculated according to Fried Wald formula[122].

LDL = TC – HDL – VLDL

VLDL cholesterol = Triglycerides / 5.

Determination of Liver Profile

Estimation of AST and ALT

Activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the method of Reitman and Frankel, 1957[123]. 0.2 ml of serum with 1 ml of substrate (aspartate and α-ketoglutarate for AST; alanine and α -ketoglutarate for ALT, in phosphate buffer pH 7.4) was incubated for an hour in case of AST and 30 minutes for ALT. 1 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 540 nm. Activities expressed as IU/L.

Estimation of ALP

Three test-tubes were set up then into the 1st (test), added 5 ml of the substrate solution (p-nitro phenyl phosphate in glycine/NaOH buffer) followed by 0.1 ml of serum[124]. After 30 minutes reaction at 37^0C, the optical density was measured at 405 nm. Into the 2nd tube, 5 ml of substrate solution was added with 0.1 ml of serum. After mixing, the optical density was measured immediately. Into the 3rd tube, 0.1 ml of water was added with 5.0 ml of p – nitro phenol standard solution, optical density was measured.

$$\text{IU/L} = \frac{\text{OD of test} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times \frac{1000}{0.1} \times \frac{1}{30} \times 10$$

<u>Determination of Renal Profile</u>

Estimation of Blood Urea

Labelled three test-tubes as B, T and S. into B, pipette, 0.02 ml water, into T, 0.02 ml blood and into S, 0.02 ml standard urea solution (40 mg urea in 100 ml of water). 0.1 ml of diacetylmonoxime solution and 5 ml of acid regent (Thiosemicarbazide) was added into all the test-tubes[125]. Mixed and kept in a boiling water bath for 15 minutes. After cooling, the absorbance was read at 540 nm and concentration of urea in mg/dl was calculated.

Estimation of Serum Creatinine

Labelled three test-tubes as B, T and S. into B, pipetted, 2 ml of water, into T, 2 ml serum and 4 ml of water, into S, 3 ml of water and 1 ml of creatinine standard (4mg/dl). Then 2 ml of ammonium sulphate and 2 ml of sodium tungstate was added in all the three test-tubes. Centrifuged and removed 3 ml of supernatant from each test tube. To that 1 ml of picric acid and distilled water was added to the supernatant of test tubes B, T and S. Absorbance was read at 520 nm and concentration of serum creatinine in mg/dl was calculated[126].

Statistical Analysis

Results were represented as mean ± SEM. The data were analysed by using one way analysis of variance (ANOVA) followed by Dunnet's't' test using graph Pad version 3. P values < 0.05 were considered as significant.

6. RESULTS

RESULTS

Effect of *Tephrosia purpurea* on Blood Sugar Levels

The antidiabetic activity of ethanolic extract of *Tephrosia purpurea* plant was studied against the STZ – Nicotinamide induced diabetes in rats and the blood sugar levels of various time intervals were shown on table 2. The blood sugar levels of diabetic control rats were higher than those of normal rats on 0, 5^{th}, 10^{th}, 20^{th} and 30^{th} day. In diabetic rats, treated with 200 and 400 mg/kg of *Tephrosia purpurea* blood glucose level significantly lowered to 157.17±5.55 on 10^{th} day ($P<0.05$) and to 189.00±5.05 on 5^{th} day ($P<0.01$) respectively as compared to diabetic animals. The diabetic rats treated with reference control glibenclamide also significantly ($P<0.001$) lowered the blood sugar level to 184.50±3.89 on 5^{th} day. On 15^{th} day onwards until the end of the drug treatment, on 30^{th} day 200 and 400 mg/kg of *Tephrosia purpurea* and glibenclamide significantly ($P<0.001$) lowered the blood glucose as compared to diabetic control animals.

Table 2. Mean ± SEM on Blood Sugar Levels

Drug Treatment	Mean Blood Sugar Level (mg/dl)						
	Before STZ + Nicotinamide	After STZ + Nicotinamide	0 Day	5th Day	10th Day	15th Day	30th Day
Control 1% CMC	105.83± 3.22	98.00± 3.91***	100.33± 4.47***	101.17± 4.67***	99.67± 4.66***	97.17± 3.03***	102.00± 5.39***
Diabetic Control	101.50± 4.59	213.00± 4.07	220.50± 5.83	228.83± 3.17	216.33± 5.83	226.50± 2.57	222.17± 5.67
Reference Control	105.17± 5.41	212.00± 4.56	219.67± 3.81	184.50± 3.89**	143.17± 2.73***	112.67± 5.57***	103.33± 5.34***
TP 200	100.67± 4.26	211.33± 4.49	219.00± 3.45	210.00± 4.07	157.17± 5.55*	129.17± 2.09***	114.17± 3.28***
TP 400	98.67± 3.45	208.83± 6.09	217.50± 4.86	189.00± 5.05**	150.83± 6.04**	119.67± 4.36***	100.17± 4.85***

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Diabetic Control

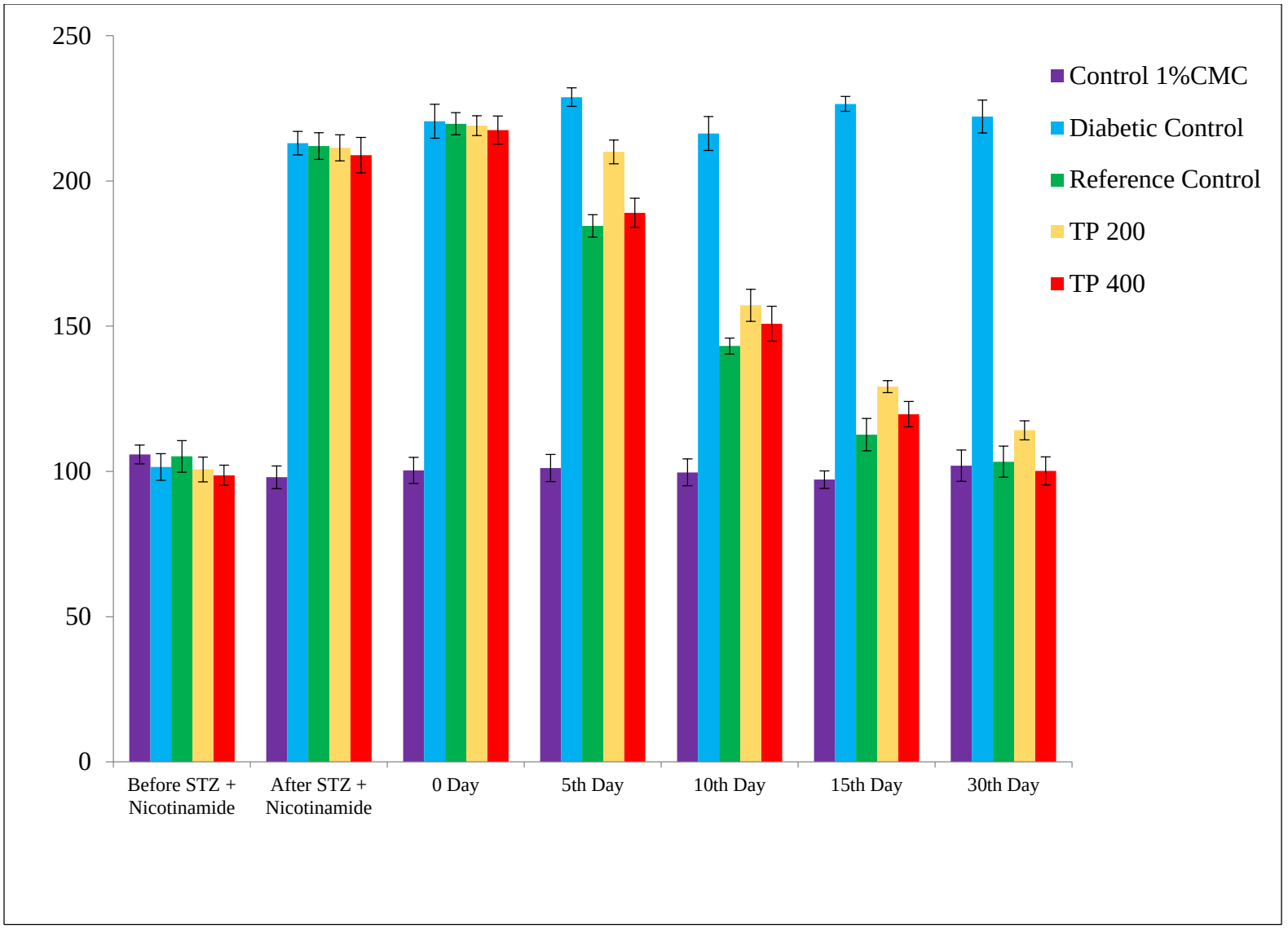

250
200
150
100
50
0
Control 1%CMC
Diabetic Control
Reference Control
TP 200
TP 400
Before STZ + Nicotinamide
After STZ + Nicotinamide
0 Day
5th Day
10th Day
15th Day
30th Day

Figure 4. Effect of Ethanolic Extract of *Tephrosia purpurea* on Blood Sugar Levels

Effect of *Tephrosia purpurea* on Lipid Profile

The effect of ethanolic extract of *Tephrosia purpurea* on lipid parameters in STZ-nicotinamide induced diabetes in rats were shown in the Table. No. 3. In the animals of normal control the total cholesterol was 112.00±2.09 mg/dl, whereas the total cholesterol was enhanced in diabetic control rats up to 138.67±1.3 3mg/dl. The 200 and 400mg/kg of ethanolic extract of *Tephrosia purpurea* significantly decreased the total cholesterol to 125.67±2.20 ($P<0.05$) and 116.00±1.41 mg/dl ($P<0.01$) respectively. The reference control Glibenclamide also significantly ($P<0.001$) reduced the total cholesterol to 113.50±1.80 mg/dl. The effect produced by the ethanolic extract of *Tephrosia purpurea* is equipotent as that of the reference control. In the animals of normal control the triglyceride was 69.33±0.98 mg/dl, whereas the triglyceride was increased in diabetic control up to 115.83±2.70 mg/dl, The 200mg/kg of ethanol extract of *Tephrosia Purpurea* significantly ($P<0.01$) decreased by reversed the elevated triglyceride to 89.17±2.24 mg/dl. *Tephrosia purpurea*, 400mg/kg and reference control glibenclamide, showed more significant ($P<0.001$) decrease in triglyceride level to 74.50±1.20 and 73.50±1.95 mg/dl respectively.

In the animals of normal control the HDL-Cholesterol was 36.67±1.28 mg/dl, whereas it was decreased in hyperlipidemic control up to 22.83±1.01 mg/dl. The 200mg/kg of ethanolic extract of *Tephrosia purpurea* showed moderate increase in HDL – Cholesterol compared to diabetic control. The

ethanolic extract of *Tephrosia purpurea* at 400mg/kg and the reference control glibenclamide significantly (P<0.001) enhanced the HDL – Cholesterol to the level of 32.67±0.49 and 31.17±1.09 respectively.

In the animals of normal control the LDL-Cholesterol was 41.00±0.63 mg/dl, where as it was increased in diabetic control up to 78.00±0.89 mg/dl. The 200mg/kg of ethanolic extract of *Tephrosia purpurea* showed significant (P<0.01) decrease in LDL – Cholesterol compared to diabetic control. The ethanolic extract of *Tephrosia purpurea* at 400mg/kg and the reference control glibenclamide showed marked and significantly (P<0.001) decrease the LDL – Cholesterol to the level of 44.00±0.97 and 46.17±0.60 respectively. VLDL – Cholesterol levels in the normal animal was 17.67±0.88 mg/dl, where as in the diabetic control it was 30.50±0.76 mg/dl. VLDL – Cholesterol (24.50±0.62 mg/dl) was significantly (P<0.05) decreased by 200mg/kg of ethanolic extract of *Tephrosia purpurea.* The levels of VLDL – Cholesterol was more significantly (P<0.01) decreased by the treatment of 400mg/kg of Tephrosia *purpurea* and the reference control glibenclamide and the levels were 21.67±0.71 and 20.17±0.70 mg/dl.

Table 3. Mean ± SEM on lipid profile

Drug Treatment	Lipid Profiles (mg/dl)				
	Total Cholesterol	Triglycerides	HDL	LDL	VLDL
Control 1% CMC	112.00± 2.09***	69.33± 0.98***	36.67± 1.28***	41.00± 0.63***	17.67± 0.88***
Diabetic Control	138.67±1.33	115.83±2.7	22.83± 1.01	78.00±0.89	30.50±0.76
Reference Control	113.50± 1.80***	73.50± 1.95***	31.17± 1.09***	46.17± 0.60***	20.17± 0.70**
TP 200	125.67± 2.20*	89.17± 2.24**	27.83± 0.91*	59.33± 0.42**	24.50± 0.62*
TP 400	116.00± 1.41**	74.50± 1.20***	32.67± 0.49***	44.00± 0.97***	21.67± 0.71**

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Diabetic Control

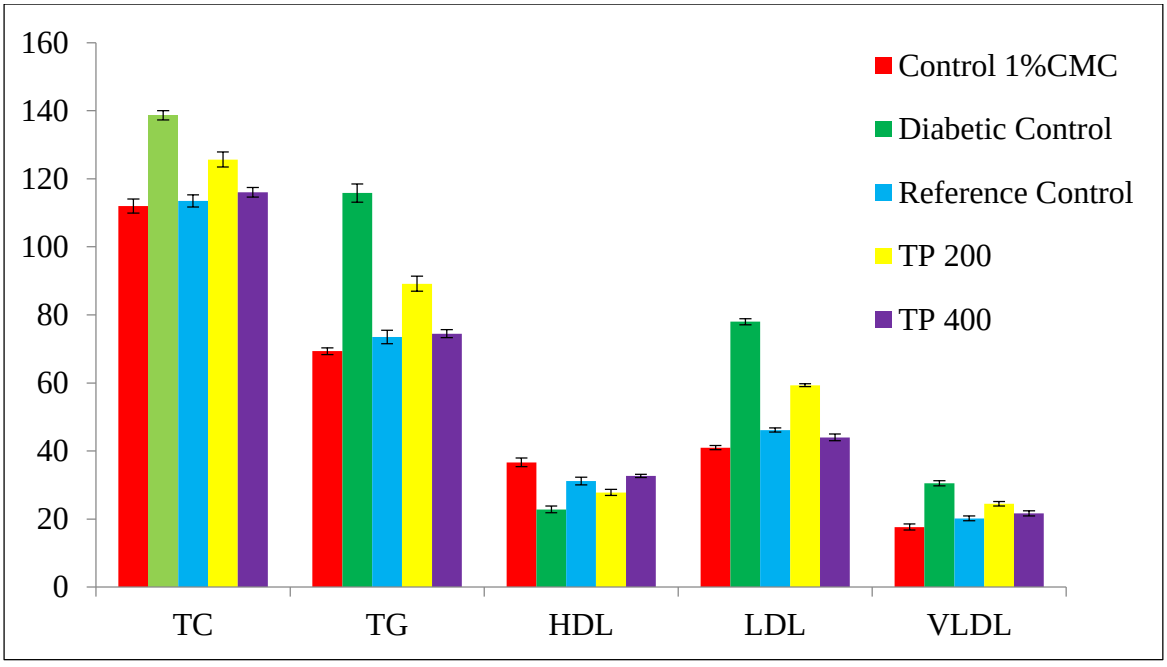


Figure 5. Ethanolic extract of *Tephrosia purpurea* on lipid profile

Effect of *Tephrosia purpurea* on Liver Enzymes and Renal Functions

The effects of ethanolic extract of Tephrosia purpurea on Liver Serum Enzymes in STZ-Nicotinamide induced diabetes in rats were shown in the Table. No 4. The activity of AST, AST and ALT were significantly elevated in diabetic control rats compared to normal controls. Administration of ethanolic extract of *Tephrosia purpurea* at 200 mg/kg, significantly ($P<0.01$) reduced serum enzymes activity of ALT, and ALP to 8.12 ± 0.48 and 45.34 ± 0.61 IU/L respectively. It also significantly ($P<0.05$) the AST to 7.24 ± 0.28 IU/L compared to diabetic control. *Tephrosia purpurea* at 400 mg/kg, significantly ($P<0.001$) reduced serum enzymes activity of ALT, and ALP to 5.59 ± 0.21 and 39.36 ± 0.52 IU/L respectively. It also significantly ($P<0.01$) the AST to 6.38 ± 0.20 IU/L compared to diabetic control. The reference control glibenclamide, showed significant ($P<0.001$) decrease in all the three serum liver enzymes as compare to diabetic control.

The effects of ethanolic extract of *Tephrosia purpurea* on serum BUN and Creatinine in STZ-Nicotinamide induced diabetes in rats and the results were shown in the Table. No 4. The Blood Urea Nitrogen and serum Creatinine were increased in the STZ – Nicotinamide induced diabetes animals as compared to normal control animals. Ethanolic extract of *Tephrosia purpurea* at 200 mg/kg, significantly ($P<0.05$) decrease the BUN (47.58 ± 0.46) and serum Creatinine (1.18 ± 0.04) as compared to diabetic control. *Tephrosia purpurea* at 400 mg/kg significantly ($P<0.001$) decrease both the BUN (32.67 ± 0.44) and serum Creatinine (0.77 ± 0.03) as compared to diabetes control.

The effect produced by Tephrosia *purpurea* 400 mg/kg on BUN and serum Creatinine was similar to that of Glibenclamide.

Table 4. Mean ± SEM on Liver and Renal Parameters

Drug Treatment	Liver Function Test			Renal Function Test	
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)
Control1% CMC	1.77 ± 0.08	4.89 ± 0.29	34.70 ± 0.58	21.65 ± 0.72	0.51 ± 0.03
Diabetic Control	13.95 ± 0.43	10.02 ± 0.37	66.49 ± 0.43	50.88 ± 0.72	1.76 ± 0.01
Reference Control	5.37 ± 0.25***	5.99 ± 0.22***	38.54 ± 0.60***	31.98 ± 0.47***	0.65 ± 0.02***
TP 200	8.12 ± 0.48**	7.24 ± 0.28*	45.34 ± 0.61**	47.58 ± 0.46*	1.18 ± 0.04*
TP 400	5.59 ± 0.21***	6.38 ± 0.20**	39.36 ± 0.52***	32.67 ± 0.44***	0.77 ± 0.03***

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Diabetic Control

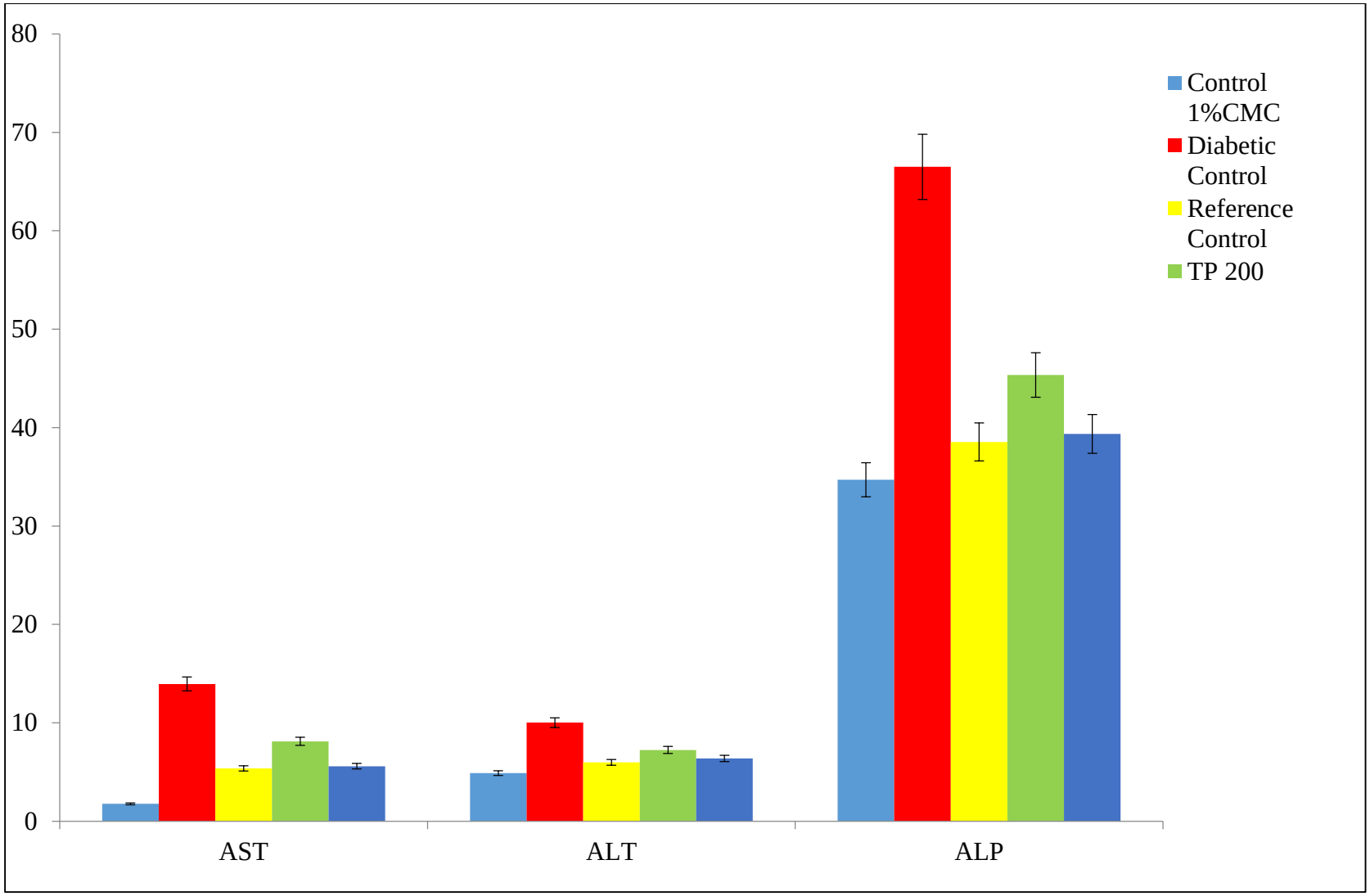


Figure 6 – Effect of ethanolic extract of *Tephrosia purpurea* on Liver function test

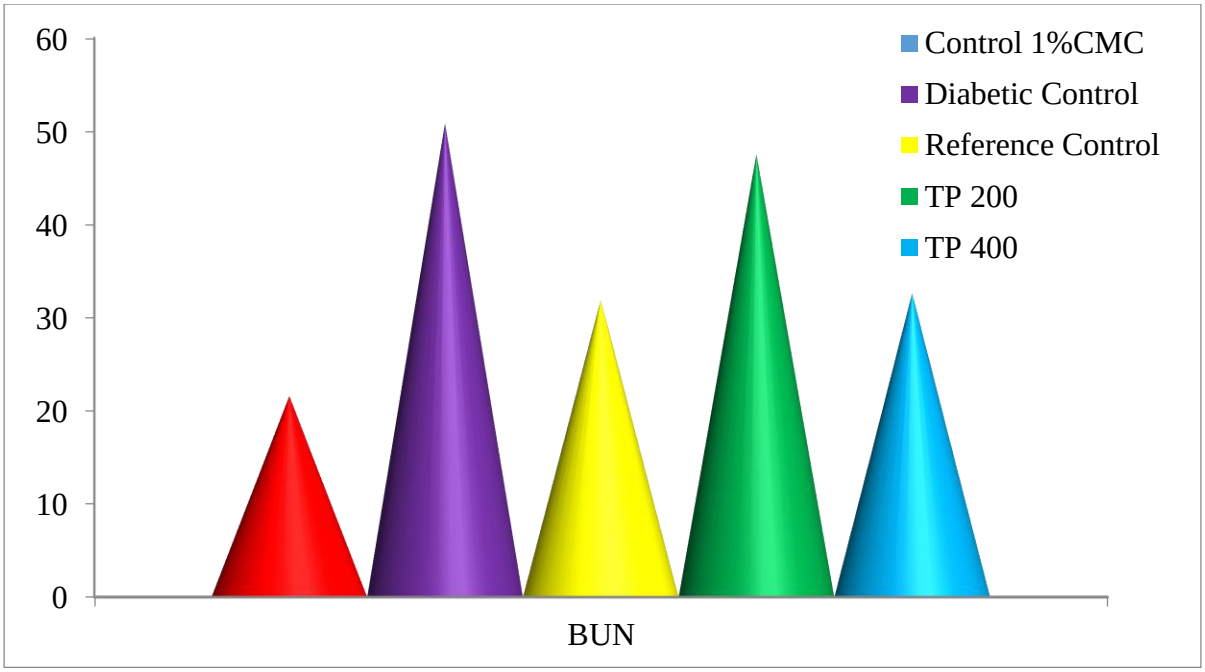


Figure 7 - Effect of ethanolic extract of *Tephrosia purpurea* on renal function test (BUN)

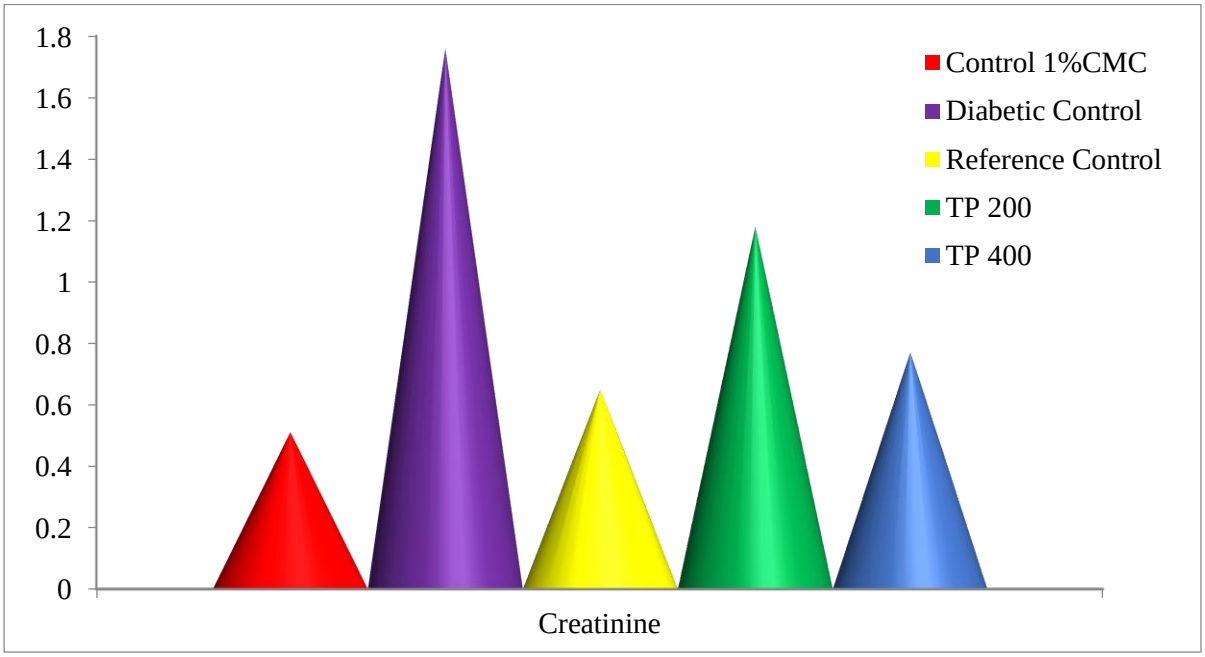


Figure 8 - Effect of ethanolic extract of *Tephrosia purpurea* on renal function test (Creatinine)

7. DISCUSSION

DISCUSSION

The present study was planned to evaluate the antidiabetic activity of ethanolic extract of *Tephrosia purpurea* against STZ – nicotinamide induced diabetes in rats. Administration of STZ and Nicotinamide has been proposed to induce experimental diabetes in rats. STZ is well known to cause pancreatic B-cell damage, whereas Nicotinamide is administered to rats to partially protect insulin-secreting cells against STZ. STZ is transported into B-cells via the glucose transporter GLUT-2 and causes DNA damage leading to increased activity of poly ADP-ribose polymerase (PARP-1). However, exaggerated activity of this enzyme results in depletion of intracellular NAD^+ and ATP, and the insulin-secreting cells undergo necrosis. The protective action of nicotinamide is due to the inhibition of PARP-1 activity. Nicotinamide inhibits this enzyme, preventing depletion of NAD^+ and ATP in cells exposed to STZ. Moreover, nicotinamide serves as a precursor of NAD^+ and thereby additionally increases intracellular NAD^+ levels. *In vitro* studies demonstrated that the insulin-secretory response to glucose is attenuated in STZ-nicotinamide induced diabetic rats compared with control animals. This is due to reduced β-cell mass as well as metabolic defects in the insulin-secreting cells[4].

The ethanolic extract of *Tephrosia purpurea* reduced blood glucose level in STZ – nicotinamide induced diabetic rats. The biochemical mechanism of actions of *Tephrosia purpurea* extract might be due to an insulin mimetic

effect by either stimulating glucose uptake and metabolism, by inhibiting hepatic gluconeogenesis and glycogenolysis[127], by stimulation of regeneration process or increase pancreatic secretion of insulin from existing β-cells and/ or inhibition activity against α-glucosidase enzymes in small intestine which convert disaccharides into monosaccharaides for sake of absorption[128].

Tephrosia purpurea at the dose of 400mg/kg exhibited significant decrease in blood glucose level, as compared to 200mg/kg on 5th day of drug administration. The result was comparable with the standard drug glibenclamide which reduced fasting blood glucose level on same day. Moreover, both the doses of *Tephrosia purpurea*, showed significant blood glucose reduction in STZ – nicotinamide induced diabetic rats on day 10th, 15th and 30th days compared to diabetic control.

Phytochemical investigations on *Tephrosia purpurea* have reported the presence of phytoconstituents such as, glycosides, carotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols[101]. It has also been suggested that, aqueous seed extract of *Tephrosia purpurea* has potent antihyperglycaemic and antioxidant effects in streptozotocin-induced diabetic rats[107]. Tremendous studies have found that flavonoids originated from foods could improve glucose metabolism, lipid profile, regulating the hormones and enzymes in human body, further protecting human being from diseases like obesity, diabetes and their complications[129]. In our findings, the antidiabetic activity exhibited by ethanolic extract of Tephrosia *purpurea* might be due to the presence of flavonoids in it.

Abnormalities in lipid profile are common complications in diabetes mellitus. Such abnormality represents the risk factors for coronary heart diseases[130]. Activation of hormone sensitive lipase during insulin deficiency causes an increase in free fatty acid mobilization from adipose tissue. In addition, hyperglycaemia is accompanied by a rise in TC, TG, LDL-C and a fall in HDL-C[131]**.** In the present study, serum total cholesterol, triglycerides, LDL-C and VLDL - C levels were decreased and at the same time HDL-C was increased in *Tephrosia purpurea* extracts treated diabetic rats.

The remarkable control of high serum triglycerides in ethanolic extract of *Tephrosia purpurea* treated diabetic rats could be due to inhibition of endogenous TG synthesis in liver or improvement in insulin level or the presence of active component(s) in *Tephrosia purpurea* that suppressed the activity of hormone sensitive lipase in adipose tissue or increased activity of hepatic lipase or lipoprotein lipase accountable for the hydrolysis of excess lipoprotein bound triacylglycerol into fatty acids[109].

Increased level of HDL-C in ethanolic extract of Tephrosia *purpurea* treated groups could be due to the enhancement of lecithin: cholesterol acyltransferase (LCAT) which plays a key role in incorporating the free cholesterol in to HDL which take back to the liver[132]. LDL-C reducing effect of *Tephrosia purpurea* presumably attributed to increased expression of low density lipoprotein receptor (LDLR), which enhance LDL particles uptake in liver from the circulation, through the depletion of intracellular cholesterol.

Serum total cholesterol lowering property of ethanolic extract of Tephrosia *purpurea* could be attributed to the presence of hypocholesterolemic compounds in *Tephrosia purpurea* that may act as inhibitor for hepatic hydroxyl methyl glutaryl CoA (HMG CoA) reductase in liver, which take part in cholesterol synthesis[109]. The decrease in serum total cholesterol, triacylglycerol, LDL-C and VLDL - C and an increase in HDL-C after 30 days treatment showed a dose dependent trend, indicating that efficacy was proportional to the dose of ethanolic extract of *Tephrosia purpurea*. In general, *Tephrosia purpurea* was capable to reverse the values of TC, TG, HDL-C and LDL-C, VLDL - C near normal after 30 days of treatment; this could be due to antioxidant property of *Tephrosia purpurea*.

The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in serum are generally indicators for liver function. In diabetic rats, the levels of these enzymes are elevated due to the necrosis of liver cells by the injection of STZ – nicotinamide. However, *Tephrosia purpurea* treated diabetic rats showed decreased in the activity of ALT, AST and ALP enzymes that might support its hepatoprotective effect and normalization capability of impaired liver metabolism in diabetic rats[111].

Negative nitrogen balance is manifested in diabetic rats associated with enhanced proteolysis in muscle and other tissues. Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentrations of urea and creatinine in serum indicates progressive renal damage in diabetic

rats[133]. Treatment with ethanolic extract of *Tephrosia purpurea* resulted in a considerable reduction to near normal in BUN and creatinine level indicating the renoprotective role of *Tephrosia purpurea* or delay diabetic nephropathy development.

8. SUMMARY

SUMMARY

This preclinical study was done to evaluate the anti-hyperglycaemic activity of whole plant extract of *Tephrosia purpurea* in STZ- Nicotinamide induced diabetic rats, which produced a significant difference in blood glucose, lipid profile, Renal and liver profile in comparison to untreated rats.

In this study, Wistar albino rats were divided into five groups containing six animals in each group. Diabetes is induced by administering STZ-Nicotinamide and fed with glucose water to prevent hypoglycaemia. Induced rats with blood sugar level >200 were enrolled in the study. Further the animals are grouped into Group I control (0.1% CMC), Group II Diseased control (STZ- Nicotinamide induced diabetes untreated rats) and treatment groups consists of Group III Glibenclamide group (STZ- Nicotinamide induced Diabetic rats treated with *glibenclamide*), Group IV and V STZ – Nicotinamide induced diabetic rats treated with *Tephrosia purpurea* extract 200mg/kg and 400mg/kg respectively. All the animals were followed up for a period of one month.

Glibenclamide is an anti-diabetic drugs belongs to 2^{nd} generation sulfonylurea, is well effective in the treatment of Diabetes mellitus is used as a reference control.

The study revealed the following findings:

- In diabetic rats, treated with 200 and 400 mg/kg of *Tephrosia purpurea* blood glucose level significantly lowered to 157.17±5.55 on 10^{th} day ($P<0.05$) and to 189.00±5.05 on 5^{th} day ($P<0.01$) respectively as compared to untreated rats. At the end of 30^{th} day there is significant reduction in blood glucose treated with TP 400mg/Kg 100.17±4.85 ($P <0.001$).
- Safety assessment shows the protective effect of TP (400mg/kg) on lipid profile TC 116±1.41 ($P <0.01$), TG 74.50 ± 1.20 ($P <0.001$), HDL 32.67±0.49 ($P<0.001$), LDL 44±0.97 ($P<0.001$) and VLDL 21.67±0.71 ($P<0.01$) .
- It also shows protective activity against AST 5.59±0.21 ($P<0.001$), ALT 6.38±0.20 ($P<0.01$), ALP 39.36±0.52 ($P<0.001$) and Renal functions BUN 32.67 ±0.44 ($P<0.001$) , Creatinine 0.77 ± 0.03 ($P<0.001$). For all safety parameters statistical values were highly significant.

The anti- hyperglycaemic activity of *Tephrosia purpurea* is brought out in the study by its significant reduction in the blood glucose level. The safety and efficacy is established based on the protective effect of *Tephrosia purpurea* in lipid profile, renal and hepatic function of diabetic rats.

9. CONCLUSION

CONCLUSION

In the present study, administration of ethanolic whole plant extract of *Tephrosia purpurea* to STZ – nicotinamide induced diabetic rats have significant reduction in blood glucose level, just about normalization of serum biochemical parameters including lipid profile (Total Cholesterol, Triglycerides, High density lipoprotein, low density lipoprotein and very low density lipoprotein), Serum liver enzymes (Alanine Aminotransferase, Aspartate Aminotransferase and Alkaline Phosphatase), Blood urea nitrogen and serum Creatinine compared to STZ – nicotinamide induced diabetic rats by similar mechanism as glibenclamide, which involves insulin sensitization effect.

Consequently, it can be concluded that, *Tephrosia purpurea* exhibited antidiabetic activity in dose dependent manner against STZ – nicotinamide induced diabetes in Wistar albino rats thereby authenticating its ethno medicinal practice. From the observed antidiabetic activity of *Tephrosia purpurea* whole plant extract against STZ – nicotinamide induced diabetic rats, the study may supports the use of it for the management of diabetes mellitus and for the prevention of further complications.

10. BIBLIOGRAPHY

BIBLIOGRAPHY

1. Alberti KGMM, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. Diabet Med. 1998; 15: 539–553.

2. Kumar A, Goel MK, Jain RB, Khanna P, Chaudhary V. India towards diabetes control: Key issues. Australas Med J. 2013; 6(10):524-31.

3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–1053.

4. Szkudelski T. Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model Exp Biol Med. 2012; 237(5): 481 – 490.

5. World Health Organization . Diabetes Mellitus: Report of a WHO Study Group. Geneva : WHO, 1985. Technical Report Series 727

6. The Expert Committee on Diagnosis and Classification of Diabetes mellitus . Report of the expert committee on the Diagnosis and classification of Diabetes mellitus. Diabetes Care 2010;20:1183-97

7. Ghalioungui P. The Ebers papyrus: A new English translation, commentaries and glossaries. Cairo: Academy of Scientific Research and Technology, 1987

8. Karamanou M, Protogerou A, Tsoucalas G, Androutsos G, Poulakou-Rebelakou E. Milestones in the history of diabetes mellitus: World J Diabetes. 2016; 7(1): 1-7.

9. Furdell EL. Fatal Thirst. Diabetes in Britain until Insulin. Leiden: Brill, 2009: 81-92

10. Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Experimental & Molecular Medicine*. 2016;48(3):e219-.

11. Von Mering J, Minkowski O. Diabetes mellitus nach Pankreas extirpation. Arch f exper Path u Pharmakol. 1889; 26: 371.

12. Cooper T, Ainsberg A. Breakthrough: Elizabeth Hughes, the Discovery of Insulin, and the Making of a Medical Miracle. New York: St Martin's Press, 2010.

13. Quianzon CCL, Cheikh IE. History of current non-insulin medications for diabetes mellitus. J Community Hosp Intern Med Perspect. 2012; 2(3):10.3402/jchimp.v2i3.19081.

14. Ramachandran A, Snehalatha C. API text book of medicine ,10th Edition. The Association of Physicians . Mumbai. Munjal Y ; 2015

15. Bailey C, Day C. Metformin: Its botanical background. Pract Diab Int.2004; 21: 115-117.

16. White JR: Overview of the medications used to treat type 2 diabetes. In Medications for the Treatment of Diabetes. White JR, Campbell RK, Eds. Alexandria. Va., American Diabetes Association. 2008; 5–15.

17. Golan David E, Tashjian Jr Armen H, Armstrong Ehrin J, Armstrong April W. Principles of Pharmacology .3rd edition. New Delhi: wolters kluwer India Pvt Ltd; 2012.

18. Global action plan for the prevention and control of non-communicable diseases 2013-2020. Geneva: World Health Organization; 2013.

19. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. AMJ 2014; 7(1):, 45-48.

20. Kasper, D., Fauci, A., Hauser, S., Longo, D., & Jameson, J. (2015). *Harrison's Principles of Internal Medicine*. New York: McGraw-Hill Education.

21. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ. Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest. 1992; 89: 283-292.

22. Banerji MA, Lebovitz HE. Insulin-sensitive and insulin-resistant variants in NIDDM. Diabet. 1989; 38: 784-792.

23. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. Int J Ped Obes. 2006; 1: 11-25.

24. Rizza RA, Gerich JE, Haymond MW. Control of blood sugar in insulin-dependent diabetes: Comparison of an artificial endocrine pancreas, continuous subcutaneous insulin infusion, and intensified conventional insulin therapy. N Engl J Med. 1980; 303: 1313-1318.

25. Consoli A, Kennedy F, Miles J, Gerich J. Determination of Krebs cycle metabolic carbon exchange in vivo and its use to estimate the individual contributions of gluconeogenesis and glycogenolysis to overall glucose output in man. J Clin Invest. 1987; 80: 1303-1310.

26. Meyer C, Nadkarni V, Stumvoll M, Gerich J. Human kidney free fatty acid and glucose uptake: Evidence for a renal glucose-fatty acid cycle. Amj J Physiol. 1997; 273: E606-E612.

27. Oster-Jorgensen E, Pedersen SA, Larsen ML. The influence of induced hyperglycaemia on gastric emptying rate in healthy humans. Scand J Clin Lab Invest. 1990; 50: 831-836.

28. Meyer C, Dostou J, Nadkarni V, Gerich J. Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. Am J Physiol Renal Physiol. 1998; 275: F915-F921.

29. Goodman, L. S., Brunton, L. L., Chabner, B., & Knollmann, B. C. (2011). *Goodman & Gilman's pharmacological basis of therapeutics*. New York: McGraw-Hill.

30. Lecavalier L, Bolli G, Cryer P, Gerich J. Contributions of gluconeogenesis and glycogenolysis during glucose counter regulation in normal humans. Am J Physiol Endocrinol Metab. 1989; 256.

31. De Feo P, Perriello G, Torlone E, Fanelli C, Ventura MM, Santeusanio F, Brunetti P, Gerich JE, Bolli GB. Contribution of adrenergic mechanisms to glucose counter regulation in humans. Am J Physiol Endocrinol Metab. 1991; 261: E725–E736.

32. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes. 1997; 46: 3-10.

33. Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus, J Physiol Pathophysiol. 2013; 4(4): 46-57.

34. Froguel P, Velho G. Genetic determinants of type 2 diabetes. Recent Prog Horm Res. 2001; 56: 91-105.

35. Polonsky KS, Sturis J, Bell GI. Non-insulin-dependent diabetes mellitus - A genetically programmed failure of the beta cell to compensate for insulin resistance. N Engl J Med. 1996; 334: 777-783.

36. Kahn SE. The importance of the β-cell in the pathogenesis of type 2 diabetes mellitus. Am J Med. 2000; 108: 2-8.

37. Leahy JL, Bonner-Weir S, Weir GC. β-Cell dysfunction induced by chronic hyperglycemia: Current ideas on mechanism of impaired glucose-induced insulin secretion. Diabetes Care. 1992; 15: 442-455.

38. Kahn SE, D'Alessio DA, Schwartz MW, Fujimoto WY, Ensinck JW, Taborsky GJ, Jr, Porte D. Jr. Evidence of cosecretion of islet amyloid polypeptide and insulin by β-cells. Diabetes 1990; 39: 634-638.

39. Clark A, Saad MF, Nezzer T. Islet amyloid polypeptide in diabetic and non-diabetic Pima Indians. Diabetologia. 1990; 33: 285-289.

40. Porte Jr D, Kahn ESE. β-cell dysfunction and failure in type 2 diabetes: Potential mechanisms. Diabetes. 2001; 50: S160-S163.

41. Charles MA, Fontbonne A, Thibult N, Warnet JM, Rosselin GE, Eschwege E. Risk factors for NIDDM in white population: Paris prospective study. Diabetes. 1991; 40: 796-799.

42. Kolterman OG, Gray RS, Griffin J. Receptor and postreceptor defects contribute to the insulin resistance in noninsulin-dependent diabetes mellitus. J Clin Invest. 1981; 68: 957-969.

43. Roden M, Stingl H, Chandramouli V. Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans. Diabetes. 2000; 49: 701-707.

44. Rossetti L, Giaccari A, DeFronzo RA. Glucose toxicity. Diabetes Care. 1990; 13: 610-630.

45. Kitabchi AE, Umpierrez GE, Fisher JN, Murphy MB, Stentz FB. Thirty years of personal experience in hyperglycemic crises: diabetic ketoacidosis and hyperglycemic hyperosmolar state. J Clin Endocrinol Metab. 2008; 93: 1541 – 1552.

46. Stentz FB, Umpierrez GE, Cuervo R, Kitabchi AE. Proinflamatory cytokines, markers of cardiovascular risks, oxidative stress, and lipid peroxidation in patients with hyperglycemic crises. Diabetes. 2004; 53: 2079 – 2086.

47. Hansen TK, Moller N. Acute Metabolic Complications of Diabetes: Diabetic Ketoacidosis and Hyperosmolar Hyperglycemia. In Textbook of diabetes 4th Edition. 2010.

48. Keller U, Szinnai G, Bilz S, Berneis K. Effects of changes in hydration on protein, glucose and lipid metabolism in man: impact on health. Eur J Clin Nutr. 2003; 57 (2): S69 – S74.

49. Moller N, Foss AC, Gravholt CH, Mortensen UM, Poulsen SH, Mogensen CE. Myocardial injury with biomarker elevation in diabetic ketoacidosis. J Diabetes Complications. 2005;19(6):361–363.

50. Gaglia JL, Wyckoff J, Abrahamson MJ. Acute hyperglycemic crisis in the elderly. Med Clin North Am. 2004; 88: 1063 – 1084.

51. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clin Diabetes. 2008; 26(2):77-82.

52. Tripathi BK, Srivastava AK, (2006). Diabetes mellitus: Complications and therapeutics. Med Sci Monit. 2006; 12(7): RA130-147.

53. Cha DR, Kim NH, Yoon JW, Jo SK, Cho WY, Kim HK and Won NH. (2000). Role of vascular endothelial growth factor in diabetic nephropathy. Kidney Inter. 2000; 58 (77): S104–S112.

54. Emanuele N, Sacks J, Klein R, Reda D, Anderson R, Duckworth W. Ethnicity, race, and baseline retinopathy correlates in the Veterans Affairs Diabetes Trial. Diabetes Care. 2005; 28: 1954 – 1958.

55. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: implications for pathogenesis. Microcir. 2007; 14: 25 – 38.

56. Bunce C, Wormald R. Leading causes of certification for blindness and partial sight in England and Wales. BMC Public Health. 2006; 6: 58.

57. Shaw JE, Zimmet PZ, Gries FA, Ziegler D. Epidemiology of diabetic neuropathy. In: Gries F.A., Cameron N.E., Low P.A. and Ziegler D. Eds. Textbook of Diabetic Neuropathy. Stuttgart/New York: Thieme. 2003; 64 – 82.

58. Abbott CA, Vileikyte L, Williamson S, Carrington AL, Boulton AJM. Multicenter study of the incidence of and predictive risk factors for diabetic neuropathic foot ulceration. Diabetes Care. 1998; 21: 1071 – 1075.

59. Sorensen L, Molyneaux L, Yue DK. (2006). The relationship among pain, sensory loss, and small nerve fibers in diabetes. Diabetes Care.2006; 29: 883–887.

60. Marchettini P, M Lacerenza, E Mauri, C Marangoni. Painful Peripheral Neuropathies. Curr Neuropharmacol. 2006; 4(3): 175–181.

61. Dash PC, Barik C, Sethy S, Dash KC. Relationship of Carotid Intima Media Thickness with Silent Cerebral Infarction in Patients of Type 2 Diabetes Nephropathy. Int J Rec Sci Res. 2017; 8(7): 18247-18252.

62. Giorgino F, Laviola L, Perin CP, Solnica B, Fuller J. Chaturvedi N. Factors associated with progression to macroalbuminuria in microalbuminuric type 1 diabetic patients: the EURODIAB prospective Complications Study. Diabetologia. 2004; 47: 1020 – 1028.

63. Chaturvedi N, Bandinelli S, Mangili R, Penno G, Rottiers RE, Fuller JH. Microalbuminuria in type 1 diabetes: rates, risk factors and glycemic threshold. Kidney Int. 2001; 60: 219 – 227.

64. Bieke F. Schrijvers, Allan Flyvbjerg, An S. De Vriese, The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. Kidney International. 65, (6), 2004, 2003-2017.

65. Holman RR, Paul SK, Bethel MA, Neil HA, Matthews DR. Long -term follow - up after tight control of blood pressure in type 2 diabetes. N Engl J Med. 2008; 359: 1565 – 1576.

66. Kissela BM, Khoury J, Kleindorfer D, Woo D, Schneider A, Alwell K. Epidemiology of ischemic stroke in patients with diabetes: the Greater Cincinnati and Northern Kentucky Stroke Study. Diabetes Care. 2004; 28: 355 – 359.

67. Beauchamp MC, Michaud SE, Li L, Sartippour MR, Renier G. Advanced glycation end products potentiate the stimulatory effect of glucose on macrophage lipoprotein lipase expression. J Lipid Res. 2004; 45: 1749 – 1757.

68. Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. Circulation. 2001; 104: 365 – 372.

69. Cooper R, Cutler J, Desvigne- Nickens P, Fortmann SP, Friedman L, Havlik R. Trends and disparities in coronary heart disease, stroke, and other cardiovascular diseases in the United States: findings of the national conference on cardiovascular disease prevention. Circulation. 2000; 102: 3137 – 3147.

70. Bertoni AG, Saydah S, Brancati FL. Diabetes and the risk of infection-related mortality in the United States. Diabetes Care. 2001; 24: 1044 – 1049.

71. Geerlings SE, Hoepelman AIM. Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunol Med Microbiol. 1999; 26: 259 – 265.

72. Cockram CS, Lee N. Diabetes and infections. In Holt RIG, Cockram CS, Flyvbjerg A, Goldstein BJ. (eds). Textbook of Diabetes, 4th Edition. John Wiley and Sons Ltd. 2010.

73. Laupland KB, Gregson DB, Zygun DA, Doig CJ, Mortis G, Church DL. Severe bloodstream infections: a population - based assessment. Cri Care Med; 2004; 32: 992 – 997.

74. Katzung Bertram G, Masters Susan B, Trevor Anthony J. Basic and Clinical Pharmacology. 13th edn. New York: Mc Graw Hill medical ; 2015.

75. Ben-Ami H, Nagachandran P, Mendelson A, Edoute Y. Drug-induced hypoglycemic coma in 102 diabetic patients. Arch Int Med. 1999; 159: 281-284.

76. Pugh JA, Wagner ML, Sawyer J, Ramirez G, Tuley M, Friedberg SJ. Is combination sulfonylurea and insulin therapy useful in NIDDM patients: A metaanalysis. Diabetes Care. 1992; 15: 953-959.

77. Holman RR. Long-term efficacy of sulfonylureas: a United Kingdom Prospective Diabetes Study perspective. Metabolism: Clinical and Experimental. 2006; 55: S2-S5.

78. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Eng J Med. 2007; 356: 2457-2471.

79. Luft D, Schmuelling RM, Eggstein M. Lactic acidosis in biguanide-treated diabetics. A review of 330 cases. Diabetologia. 1978; 14: 75-87.

80. Hamilton MT, Booth FW. Skeletal muscle adaptation to exercise: A century of progress. J Appl Physiol. 2000; 88: 327-331.

81. Swanston-Flatt SK, Flatt PR, Day C, Bailey CJ. Traditional dietary adjuncts for the treatment of diabetes mellitus. Proceed Nutr Soci. 1991; 50: 641-651.

82. Bailey CJ, Turner RC. Metformin. N Eng J Med. 1996; 334: 574-579 .

83. Iranloye BO, Arikawe AP, Rotimi G, Sogbade AO. Anti-diabetic and anti-oxidant effects of Zingiber officinale on alloxan-induced and insulin-resistant diabetic male rats. Nigerian journal of physiological sciences : official publication of the Physiological Society of Nigeria 2011;26(1):89-96.

84. Herr RR, Jahnke JK, Argoudelis AD. The structure of streptozotocin. J Am Chem Soci. 1967;89(18):4808-9.

85. Mythili MD, Vyas R, Akila G, Gunasekaran S. Effect of streptozotocin on the ultrastructure of rat pancreatic islets. Micro Res Tech. 2004;63(5):274-81.

86. Epand RM, Stafford AR, Tyers M, Nieboer E. Mechanism of action of diabetogenic zinc-chelating agents. Model system studies. Mol Pharmacol. 1985;27(3):366-74.

87. Karasawa H, Takaishi K, Kumagae Y. Obesity-induced diabetes in mouse strains treated with gold thioglucose: a novel animal model for studying β-cell dysfunction. Obes. (Silver Spring, Md.) 2011;19(3):514-21.

88. Nagata M, Suzuki W, Iizuka S, Tabuchi M, Maruyama H, Takeda S. Type 2 diabetes mellitus in obese mouse model induced by monosodium glutamate. Exp Ani Jap Asso Lab Ani Sci. 2006;55(2):109-15.

89. Gamble DR, Kinsley ML, FitzGerald MG, Bolton R, Taylor KW. Viral antibodies in diabetes mellitus. Bri Med J. 1969;3(5671):627-30.

90. Utsugi T, Kanda T, Tajima Y, Tomono S, Suzuki T, Murata K. A new animal model of non-insulin-dependent diabetes mellitus induced by the NDK25 variant of encephalomyocarditis virus. Diabetes Res (Edinburgh, Scotland) 1992;20(4):109-19.

91. Horwitz MS, Bradley LM, Harbertson J, Krahl T, Lee J, Sarvetnick N. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. Nature medicine 1998;4(7):781-5.

92. Campbell J, Chaikof L, Davidson IWF. Metahypophyseal diabetes produced by growth hormone. Endocrinol 1954:48-58.

93. Heather A, Ferris C, Kahn R. New mechanisms of glucocorticoids-induced insulin resistance: make no bones about it. J Clin Invest. 2012;122: 3854-57.

94. Gould CL, McMannama KG, Bigley NJ, Giron DJ. Virus-induced murine diabetes. Enhancement by immunosuppression. Diabetes. 1985;34(12):1217-21.

95. Nadkarni KN, Nadkarni AK, The indian material medica, popular prakashan,1,200, 561.

96. Warrier PK, Nambiar VPK, Ramankutty C. Indian Medicinal Plants. Vol-1.Oriant Longman Ltd; Arya Vaidya Sala: Kottakkal, 2000; 249 - 251.

97. Sharma R, Mehan S, Kalra S, Khanna D *Tephrosia purpurea* – A Magical Herb With Blessing In Human Biological System. Int J Recent Advan In Pharmal Res. 2013; 3(3): 12-22.

98. Chopra RN, Nayer SL, Chopra IC "Glossary of Indian Medicinal Plants", Council of Scientific and Industrial Research, New Delhi, India. 1956-92; I: 241.

99. Deshpande SS, Shah GB. "Pharmacological activity of *Tephrosia purpurea",* Am Asso Pharm Scient J, 2003;10: 55-59.

100. Kumari S, Srivastava M, Abbasi P. Response of *Tephrosia purpurea* to salinity stress in relation to germination, carotenoid content and proline content. An Int Quart J Biol Life Sci. 2014; 2: 276-281.

101. Chaudhari TB, Tambe DA, Chaudhari SR. Phytopharmacology of *Tephrosia purpurea Pers.* (Fabaceae). IJPI's J Pharmacog Herb Form. 2012; 2(8):1-13.

102. Chang LC, Chavez D, Song LL, Farnsworth NR, Pezzuto JM, Kinghorn AD. Absolute configuration of novel bioactive flavonoids from *Tephrosia purpurea* .Org Lett. 2000; 2: 515- 518.

103. Venkata Rao E, Ranga Raju N. Two flavonoids from *Tephrosia purpurea.* Phytochem. 1984;23(10): 2339- 2342.

104. Gupta RK, Krishnamurti M, Parthasarathi J. A new flavanone from *Tephrosia purpurea* seeds. Phytochem. 1980; 19: 1264.

105. Pavana P, Manoharan S, Renju GL, Sethupathy S.Antihyperglycemic and antihyperlipidemic effects of *Tephrosia purpurea* leaves extract in streptozotocin induced diabetic rats. J Environ Biol. 2007; 28(4):833-837.

106. Hutchings A, van Staden J. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1: Plants used for headaches. J Ethnopharmacol. 1994; 43(2):89-124.

107. Pavana P, Sethupathy S, Santha K, Manoharan S. Effects of *Tephrosia purpurea* aqueous seed extract on blood glucose and antioxidant enzyme activities in streptozotocin induced diabetic rats. Afr J Trad CAM. 2009; 6 (1): 78-86.

108. Pavana P, Sethupathy S, Manoharan S. Antihyperglycemic and antilipidperoxidative effect of TP seed extract in streptozotocin induced dibetic rats. Ind J Clin Biochem. 2007; 22 (1) 77-83.

109. Sayad mustak. Comparative study of *Tephrosia purpurea* (Linn) leaves and Lovastatin on cholesterol level of hyperlipidemic wistar rats. IOSR J Pharm Biol Sci. 2012;1(2):25-30.

110. Sangeetha B, Krishnakumari S. Folk Medicinal plant *Tephrosia purpurea* ameliorates carbon tetrachloride induced hepatic damage in rats. Int J Pharma Biosci.2010;1(2):1-10.

111. Khatria A, Gargb A, Shyam S Agrawal. Evaluation of hepatoprotective activity of aerial parts of *Tephrosia purpurea* L. and stem bark of *Tecomella undulate.* J Ethnopharmacol. 2009; 122: 1–5.

112. Manjula RR, Spandana U, Joshi Anand T, Sudheer M. *In vitro* anthelmintic activity of aqueous and methanolic leaf extract of *Tephrosia purpurea* linn. Int J Res Pharm Chem. 2013;3(1):12-14.

113. Ashok kumar D, Narayana TV, Vidyasagar, Mazumder UK, Gupta M. Exploration of diuretic potential and electrolyte excretion of *Tephrosia purpurea* (Fabaceae) in rats. J Diet Sup. 2012;9(1):9-18.

114. Gopalakrishnan S, Vadivel E, Dhanalakshmi K. Antiinflammatory and analgesic activities of *Tephrosia purpurea* Linn. Aerial and root extracts. J Pharm Res. 2010; 3(5): 1103-1106.

115. Rumit Shah, Heena Kathad, Rajal Sheth, Naveen Sheth. *In vitro* antioxidant activity of roots of *Tephrosia purpurea* linn. Int J Pharm Pharmaceu Sci. 2010; 2(3): 30-33.

116. Damre AS, Gokhale AB, Phadke AS, Kulkarni KR, Saraf MN. Studies on the immunomodulatory activity of flavonoidal fraction of *Tephrosia purpurea* . Fitoterap. 2003; 74: 257-261.

117. Soria B, Quesada I, Ropero AB, Pertusa JA, Martin F, Nadal A. Novel players in pancreatic islet signaling: from membrane receptors to nuclear channels. Diabetes. 2004;53 (Suppl 1):S86–91.

118. Hatou S, Yamada M, Akune Y, Mochizuki H, Shiraishi A, Joko T, Nishida T, Tsubota K. Role of insulin in regulation of Na+-/K+-dependent ATPase activity and pump function in corneal endothelial cells. Invest Ophthalmol Vis Sci. 2010 ;51(8):3935-42

119. Roeschlau P, Bernt E, Gruber WA Enzymatic determination of total cholesterol in serum. Clin Biochem. 1974; 12: 226-228.

120. Tietz NW. Clinical guide to laboratory tests. 2nd ed. W.B. Saunders Company, Philadelphia. 1990.

121. Lopes-Virella MF, Stone P, Ellis S, Colwell JS. Cholesterol determination in high density lipoproteins separated by three different methods. Clin Chem. 1977; 23: 882-884.

122. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972; 18: 499-502.

123. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957; 28: 56 - 63.

124. King EJ. Assay of acid and alkaline phosphatase. In Practical Clinical Enzymology (D. Van Nostrand Co., London). 1965; 85-87.

125. Natelson S, Scott ML, Beffa C. A rapid method for the estimation of urea in biological fluid by means of the reaction between diacetyl-monoxime and urea. Am J Chem Pathol. 1951; 21: 275.

126. Slot C. Plasma creatinine determination: a new and specific Jaffe reaction method. Scand J Clin Invest. 1965; 17: 381.

127. Daisy P, Balasubramanian K, Rajalakshmi M, Eliza J, Selvaraj J. Insulin mimetic impact of Catechin isolated from *Cassia fistula* on the glucose oxidation and molecular mechanisms of glucose uptake on Streptozotocin induced diabetic Wistar rats. Phytomed. 2010;17: 28-36.

128. Shinde J, Taldone T, Barletta M, Kunaparaju N, Hu B, Kumar S, Placido J, Zito SW. α-Glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel in vitro and in Goto–Kakizaki (GK) rats. Carbohydrate Res. 2008; 343**:** 1278-1281.

129. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: A cellular mechanism review. Nutr Metab. 2015; 12:60.

130. Shamaony LA, Khazraji SMA, Twaij HAA. Hypoglycaemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. J Ethnopharmacol. 1994; 43**:** 167-171.

131. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nature Clin Prac Endocrinol & Metab. 2009; 5, 150-159.

132. Ghatak SB, Panchal SJ. Anti-hyperlipidemic activity of oryzanol, isolated from crude rice bran oil, on Triton WR-1339-induced acute hyperlipidemia in rats. Braz J Pharmacog. 2012; 23, 642-648.

133. Rumit S, Kathad H, Sheth R, Sheth N. *In vitro* antioxidant activity of roots of *Tephrosia purpurea* linn. Int J Pharm Pharmaceu Sci. 2010; 2(3), 30-33.

11. ANNEXURES

ANNEXURE I

KARPAGA VINAYAGA INSTITUTE OF MEDICAL SCIENCES & RESEARCH CENTER,
GST ROAD, PALAYANOOR POST, MADURANTHAGAM-603 308
Ph:044-27565195/27598484, email-kims.medical@yahoo.com

16.04.2016

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

Date of Meeting: 16.04.2016 **Venue : Lecture Hall, KIMS**

1. Registration Number of the Institution : **1818/GO/Ere/S/15/CPCSEA**
2. Proposal Number : 008/IAEC/KIMS/2016
3. Title of the Project : experimental evaluation of oral anit- Anti-diabetic activity of whole plant extract of tephrosia purpurea in STZ-Nicotinamide induced diabetes rats.
4. Principle Investigator and Co- PI : Dr. Deepti
5. Date first Received :16.04.2016
6. Date received after modification : NA
7. Dater received after second : NA
8. Approval date : 16.04.2016
9. No.of Animals Sanctioned after meeting : **48nos.**

Dr. R. Ilavarasan
CPCSEA Nominee
Assistant Director (S-3) In-charge
Captain Srinivasamurti Research Institute for
Ayurveda and Siddha Drug Development
A.A. Government Hospital Campus
Arumbakkam, Chennai-600 106.

Dr. Girija Sivakumar
Secretary,IAEC,KIMS
Professor of Anatomy
Karpaga Vinayaga Institute
of Medical Sciences
Madhuranthagam

ANNEXURE II

URKUND

Urkund Analysis Result

Analysed Document: Dissertation final 2 reference.docx (D31017831)
Submitted: 10/4/2017 5:18:00 PM
Submitted By: deeptidennison@gmail.com
Significance: 7 %

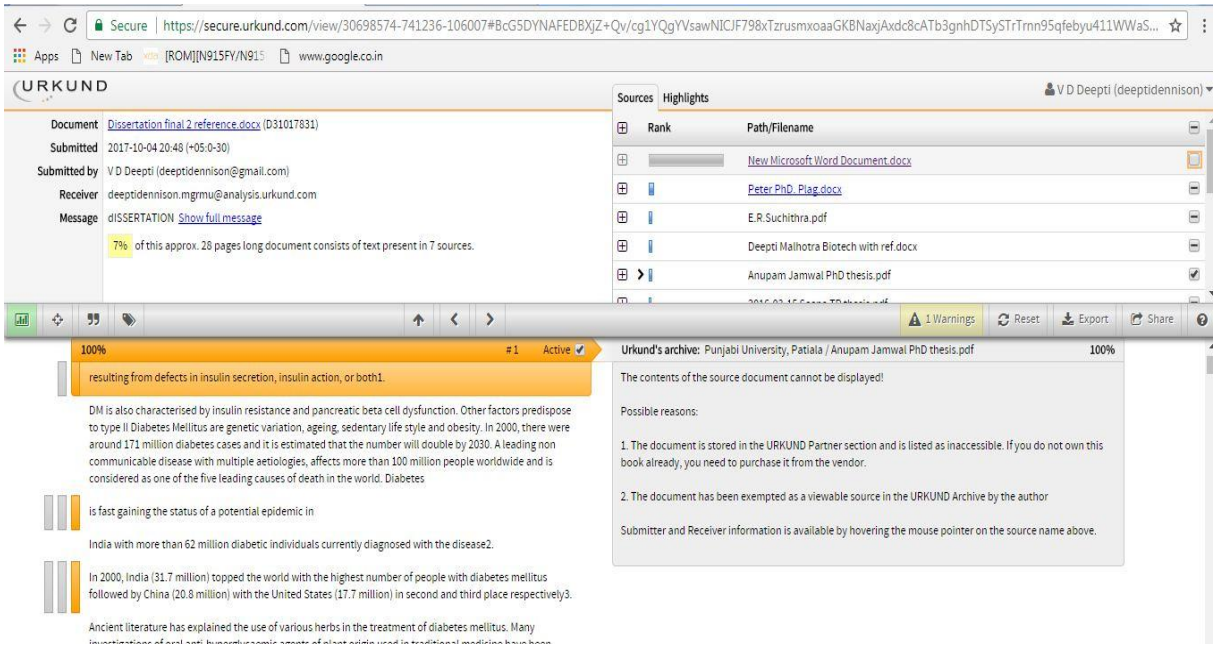
Sources included in the report:

Deepti Malhotra Biotech with ref.docx (D22976858)
2016-03-15 Seena TP thesis.pdf (D18555289)
Anupam Jamwal PhD thesis.pdf (D27257091)
E.R.Suchithra.pdf (D23683072)
Peter PhD. Plag.docx (D27214860)
http://bioinfo.bisr.res.in/project/domap/plant_details.php?plantid=0123&bname=Tephrosia%20purpurea
http://www.ijpsr.info/docs/IJPSR-10-01-01-10.pdf

Instances where selected sources appear:

18

ANNEXURE II A



ANNEXURE III

NANDHA COLLEGE OF PHARMACY

(Approved by Govt. of Tamilnadu, AICTE, New Delhi, Recognized by Pharmacy Council of India, New Delhi,
Affiliated to The Tamilnadu Dr. M.G.R.Medical University, Chennai)

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Web : www.nandhainstitutions.org E-mail : nandha_pharmacy@yahoo.co.in

Dr .R.Duraisami,
Professor & Head Dept. of Pharmacognogy

Date **16-09-2016**

No: NCP/Phcog/2016/0202

To

Dr. V.D. Deepti,
Department of Pharmacology,
Karpaga Vinayaga Institute of Medical Sciences,
Madhuranthagam,
Kancheepuram District.

Dear Sir/Madam,

The plant specimen brought and submitted for the identification is identified as *Tephrosia purpurea* belonging to the family- Leguminosae (Fabaceae).

Thanking you,

Yours faithfully,

(Dr. R. Duraisami)

Nandha College of Pharmacy Erode-52